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Original Paper

Portal Cytokine Response and Metabolic Markers in the Early Stages of Abdominal Sepsis in Pigs

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Key Words
Cytokines · *Escherichia coli* · Peritonitis · Portal vein · Sepsis

Abstract
Background: The portal vein could play a major role in disseminating the local inflammation of acute bacterial peritonitis since it is responsible for the venous drainage of the gastrointestinal tract. We hypothesized that after peritoneal exposure to *Escherichia coli*, a gradient between the portal and systemic levels of cytokines would be expected. ***Methods:*** Acute peritonitis was induced by depositing 200 ml of broth with live *E. coli* in the peritoneal cavity of the animals in the B-group (n = 7). They were then observed for 4 h and compared with a control group (C-group, n = 7). Tumour necrosis factor alpha (TNF-α), interleukin (IL)-6, IL-10 and vascular endothelial growth factor were measured repeatedly in the portal vein and the femoral artery. Portal vein metabolic markers (microdialysis), haemodynamics, biochemistry, plasma volume (PV), fluid shifts and total tissue water content were recorded or calculated. ***Results:*** The intervention led to PV contraction, increased fluid extravasation, increased pulmonary vascular resistance and reduced urinary output in the B-group as compared with the C-group. The levels of glucose in the portal vein were reduced in both study groups with no between-group differences. The levels of TNF-α and IL-6 increased markedly in the portal vein as well as in the systemic circulation of the B-group, but no gradient was seen between them. The corresponding levels of TNF-α and IL-6 remained low and stable in the C-group. ***Conclusion:*** The portal vein appears to play a minor role in supplying TNF-α and IL-6 to the systemic circulation after peritoneal exposure to a substantial dose of *E. coli*.

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Introduction

Bacterial peritonitis frequently follows exposure of the abdominal cavity to Gram-negative bacteria, triggering a systemic inflammatory response occasionally leading to multiple organ failure. The initial peritoneal response includes activation of resident macrophages and mesothelial cells, resulting in phagocytosis and recruitment of polymorphonuclear granulocytes and monocytes into the peritoneal cavity by chemotaxis [1]. The systemic dissemination may be mediated by microbial products or inflammatory mediators transported to the systemic circulation by the venous system or lymphatic drainage [2]. The portal vein is responsible for the venous drainage of the visceral peritoneum and since the latter constitutes about 80% of the total peritoneal surface, the portal vein may play an important role in triggering a systemic inflammatory response [3].

Pro-inflammatory cytokines are immediately generated by mesothelial cells and resident macrophages [1]. Intra-abdominal levels of pro-inflammatory cytokines in bacterial peritonitis have been shown to exceed by far the systemic levels, and this phenomenon has been explained by the concept of compartmentalisation, meaning that the peritoneal and the systemic inflammatory response occur in two functionally separate compartments [4, 5].

In the present study, we repeatedly compared the levels of systemic and portal cytokines 4 h after depositing live *Escherichia coli* in the abdominal cavity of pigs. We aimed to test the hypothesis that the portal vein is a major route for the transportation of cytokines from the abdominal cavity to the systemic circulation after excessive exposure to *E. coli*. To assess the clinical relevance of the experimental model, we also monitored the local and systemic physiologic response to the intervention.

Materials and Methods

Animal Handling

Fourteen immature domestic pigs (Norwegian landrace – Yorkshire hybrid) were studied. The study protocol was approved by the Institutional Veterinarian Authority (October 29, 2009; No. 20092193) prior to the beginning of the study. Animal handling was in accordance with the recommendations given by the Norwegian State Commission for Laboratory Animals. They were all acclimatised to the facilities for 5 days before the experiments were started. Food was withdrawn 8–12 h prior to anaesthesia, and water was provided at all times.

Ketamine 500 mg, diazepam 10 mg and atropine 1 mg was given as pre-anaesthetic medication. Induction of general anaesthesia was achieved by inhalation of isoflurane in oxygen, supplemented with 5 mg/kg of intravenous (iv) thiopentone prior to intubation of the trachea (blue line oral tube, ID 6.0; Mallinckrodt, Hazelwood, Mo., USA). All animals were submitted to volume-controlled ventilation (Julian anaesthesia work station; Dräger, Lübeck, Germany), and general anaesthesia was maintained by inhalation of isoflurane 0.5–2% in 40–60% oxygen in air, supplemented with a continuous iv infusion of midazolam 0.5 mg/kg/h and fentanyl 7.5 μg/kg/h [6].

All animals were given continuous iv infusion of acetated Ringer's solution from induction of anaesthesia. Blood samples and bleeding were substituted by acetated Ringer's solution in volumes three times the volume of blood lost from the circulation.

At the end of the experiments, the animals were sacrificed by an iv injection of a 20-ml saturated KCl solution.

Surgical Procedures and Haemodynamic Monitoring

The femoral artery and vein was surgically exposed and catheters were introduced into the respective vessels (Secalone-T™, 18 G; BD Medical, Singapore). Mean arterial and femoral central venous pressures were continuously monitored by fluid-filled lines connected to transducers (Transpac® II; Abbot Critical Care Systems, Sligo, Republic of Ireland) linked to a monitor (HP-78353A; Hewlett Packard, Palo Alto, Calif., USA). The right external jugular vein was surgically exposed, and a Swan-Ganz catheter was introduced and posi-

tioned in the pulmonary artery for recording of the mean pulmonary artery pressure, pulmonary capillary wedge pressure and cardiac output (CO) by the thermodilution technique (5F Swan-Ganz Thermodilution Catheter; Baxter Healthcare Corporation, Irvine, Calif., USA).

The abdomen was entered through a midline incision. A two-lumen central venous catheter was introduced into the portal vein for collecting blood samples and recording portal venous pressure (Certofix Duo 16 G, 15 cm; B Braun, Melsungen AG, Melsungen, Germany). In parallel, a microdialysis catheter (CMA 63, 30 mm, cut-off: 20,000 Dalton; CMA Microdialysis AB, Solna, Sweden) was placed in the portal vein through an 18 G iv cannula (Venflon™ Pro; BD, Helsingborg, Sweden). The urinary bladder was punctured, and a Foley catheter was introduced for monitoring of the urinary output. A triple lumen central venous catheter was placed in the abdominal cavity for infusion of broth and pressure monitoring (16 cm, 12 French 3-lumen catheter, Arrow-Howes™ Large-Bore Multi-Lumen CVC-Set; Arrow International, Inc., Reading, Pa., USA). The catheter tip was placed 10 cm to the left of the midline. All catheters were brought out through the midline incision. The abdomen was then closed in layers with a running stitch.

Study Protocol

Following surgical preparation, all animals were stabilised for 60 min before the intervention. They were randomised either to receive an intra-abdominal infusion of *E. coli* in broth (B-group, n = 7) or to receive the same amount of broth without *E. coli* (C-group, n = 7). Randomisation was performed by a microbiologist prior to the experiments, and the allocation of each animal was not revealed to the rest of the research team before all experiments were completed. After the intervention, the animals were observed for 4 h before they were sacrificed.

Microbiology

E. coli ATCC 25922™ was grown over night on blood agar with 5% sheep blood before it was inoculated in 200 ml of Mueller Hinton (MH) broth cultured for 4 h at 37°C and administered to the animal. The B-group received 200 ml of MH broth with *E. coli*, while the C-group received 200 ml MH broth without *E. coli*. The broth was shaken every 30 min during culturing. In separate tests, the optimal incubation time for *E. coli* in MH broth was found to be 4 h. After 4 h of incubation, the number of colony forming units (CFU)/ml decreased. The concentration of organisms in the broth was determined by using the DensiChek densitometer (Biomérieux, Marcy L'Étoile, France) and by counting CFU on blood agar with 5% sheep blood.

Inflammatory Mediators

Blood samples from the femoral artery and the portal vein were collected every 30 min. Serum levels of tumour necrosis factor alpha (TNF-α), interleukin (IL)-6 and IL-10 were measured by a quantitative sandwich enzyme immunoassay technique (Quantikine Porcine Immunoassay; R&D Systems, Minneapolis, Minn., USA) according to the manufacturer's protocol. The reported mean minimum detectable doses of IL-10, IL-6 and TNF-α were 3.5 pg/ml, 2.03 pg/ml and 3.7 pg/ml, respectively. Serum vascular endothelial growth factor (VEGF) was measured by a quantitative sandwich enzyme immunoassay technique (GSI Porcine VEGF Elisa Kit; Genorise Scientific, Inc., Paoli, Pa., USA), and assay sensitivity was reported to be 0.7 pg/ml.

Microdialysis

The microdialysis catheter in the portal vein was perfused by acetated Ringer's solution with a flow rate of 0.3 μl/min by a microinfusion pump (CMA 107). Samples of microdialysate were collected in microvials, and glucose, lactate and pyruvate were analysed every 30 min by a CMA 600 microanalyzer [7].

Plasma Volume, Net Fluid Balance and Fluid Extravasation Rate

After 30 min of stabilisation, the erythrocyte volume of the animal was determined by an indicator dilution technique using carbon monoxide as an indicator. The method has previously been presented in detail [8]. Based on repeated measurements of haematocrit (Hct) and blood loss during the experiments, new values for the erythrocyte volume were calculated every 30 min. Hence, plasma volume (PV) at baseline and during the experiments could also be easily calculated.

Net fluid balance (NFB) was expressed as the total amount of fluid added minus the recorded loss of fluid over a defined period of time, usually 30 min. Fluid extravasation (FE) was defined as NFB minus the change in PV during the same time interval. The resulting fluid extravasation rate (FER) was presented as ml/kg/min.

FE (ml/kg) = NFB (ml/kg) – ΔPV (ml/kg)

Blood Chemistry

Blood samples were drawn from the arterial line for determination of the haemoglobin concentration, Hct, serum albumin, serum total protein, serum electrolyte concentrations, serum osmolality as well as acid base parameters. Hct was determined using standard Hct tubes centrifuged at 12,000 rpm for 10 min. Serum albumin and serum total protein concentrations were analysed in an automatic analyser, as were serum electrolytes (Clinical Analyzer 7600, Modular P; Hitachi High-Technologies Corporation, Tokyo, Japan). Serum osmolality was analysed by freezing point determination (Fiske 110 osmometer; Fiske Associates, Norwood, Mass., USA).

Total Tissue Water

Immediately after sacrificing the animal, tissue samples were taken from the left quadriceps muscle, abdominal skin, colon, ileum, stomach, liver, pancreas, kidney, lung and heart (left and right ventricular myocardium) and placed in pre-weighed vials, reweighed and placed in a drying chamber at 70°C. The vials were weighed repeatedly until stable weight was achieved. The weight reduction was considered to represent the water content of the samples. Total tissue water was recorded as g/g dry weight.

Statistics

Analysis of variance for repeated measurements with one grouping variable was used to test the outcome variables at different points in time. When a significant between-group difference or a significant interaction between the study groups was found, independent t tests or Mann-Whitney U tests were performed to compare the groups at corresponding time points. The Mann-Whitney U test was used as a post-test when the data was not normally distributed.

If a within-group difference was found, paired t tests were performed to compare the values of stabilisation after 60 min with the values obtained 120 and 240 min after the introduction of broth into the peritoneal cavity. The urinary output in the two study groups during the intervention was compared with the Mann-Whitney U test.

All results are presented as means with SD in parentheses. The level of statistical significance was set at $p < 0.05$, and the p value was adjusted according to the numbers of comparisons performed. The analyses were conducted by the statistical software package PASW Statistics 18 (2009) for Windows.

Results

The age of the animals in the C-group and the B-group was 94.0 (17.2) and 101.9 (16.2) days and they weighed 32.8 (2.7) and 33.7 (2.3) kg, respectively ($p > 0.05$). The female/male ratio was 2/5 in the C-group and 3/4 in the B-group. One animal of the B-group had circulatory arrest 20 minutes before completion of the protocol. Therefore, the data recorded 240 min after the intervention is based on 6 animals in the B-group and 7 animals in the C-group.

The samples of the broth were cultured before use. The B-group received 200 ml of broth with $4.6\ (3.5) \cdot 10^7$ CFU/ml. The peritoneal surface was found to be partly covered by fibrin in the animals of the B-group in the post mortem examination.

The haemodynamic results are presented in table 1 and figure 1. The mean arterial pressure decreased in both study groups during the experiments, while the mean pulmonary artery pressure increased in the B-group and was higher than the value of the C-group after 240 min ($p < 0.05$). Femoral and portal venous pressures remained essentially unchanged, while the intra-abdominal pressure showed a slight but significant increase in both groups. The systemic vascular resistance index remained stable in both groups, while the pulmonary vascular resistance index increased in the B-group and differed from the C-group at the end of the experiments ($p < 0.05$). CO decreased from 3.8 (0.7) to 3.0 (0.5) litre/min in the C-group and from 3.7 (0.7) to 2.7 (0.7) litre/min in the B-group. There was a trend to lower values in the B-group, but there were no statistically significant differences between the groups.

Fluid shifts and the change in PV (%) are depicted in figure 2. NFB was similar in the two groups throughout the study.

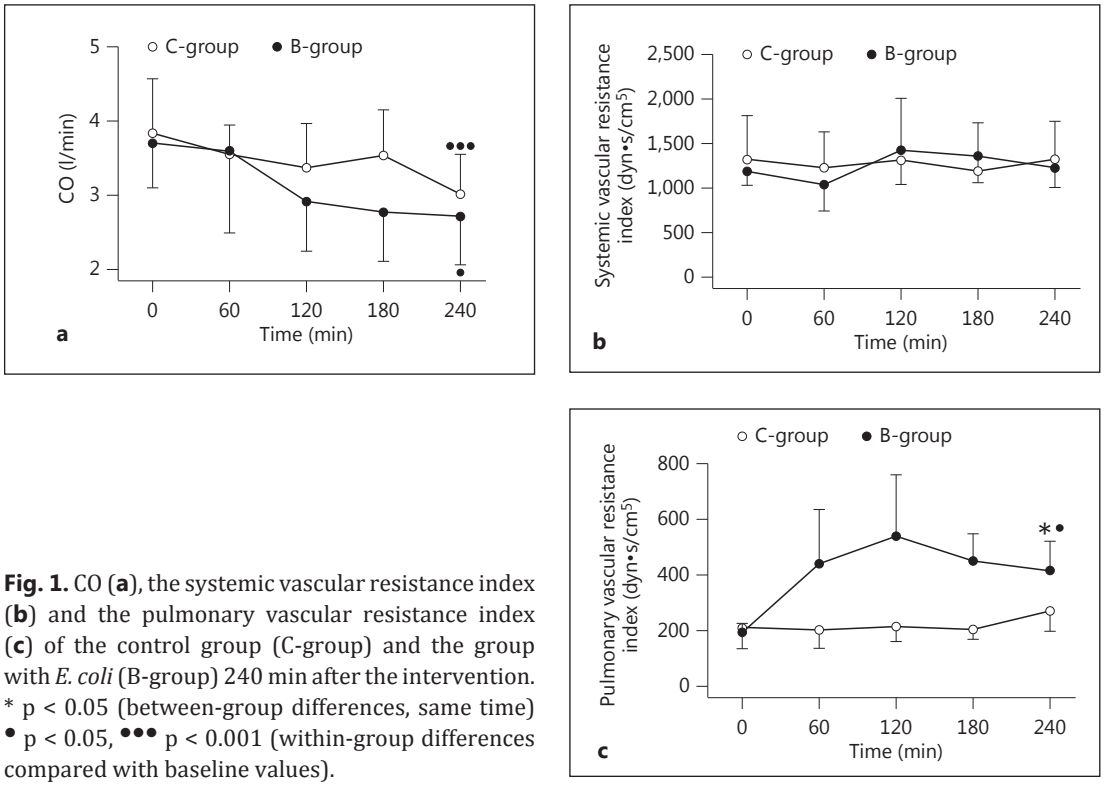

Fig. 1. CO (**a**), the systemic vascular resistance index (**b**) and the pulmonary vascular resistance index (**c**) of the control group (C-group) and the group with *E. coli* (B-group) 240 min after the intervention. * $p < 0.05$ (between-group differences, same time) • $p < 0.05$, ••• $p < 0.001$ (within-group differences compared with baseline values).

Table 1. Haemodynamic and fluid parameters

	Group	Baseline	60 min	120 min	180 min	240 min
MAP, mm Hg	C-group	73.9 (15.2)	66.3 (14.6)	63.3 (14.5)	61.9 (14.5)	59.6 (10.5)[a]
	B-group	65.5 (6.6)	53.3 (7.4)	58.8 (9.3)	53.3 (8.3)	51.3 (8.0)[a]
MPAP, mm Hg	C-group	21.4 (3.0)	22.1 (4.4)	22.7 (6.0)	22.6 (5.8)	24.1 (7.3)
	B-group	19.9 (2.4)	29.4 (6.0)	29.9 (8.6)	31.0 (8.5)	31.1 (5.9)[b, *]
CVP_F, mm Hg	C-group	7.9 (2.3)	8.3 (2.4)	8.1 (2.9)	8.3 (2.8)	8.0 (3.3)
	B-group	8.2 (1.8)	7.7 (2.6)	8.3 (1.6)	7.7 (2.9)	8.3 (2.8)
PVP, mm Hg	C-group	9.3 (3.0)	10.1 (4.6)	9.7 (5.5)	10.3 (5.5)	9.0 (3.6)
	B-group	8.3 (2.2)	8.8 (2.7)	9.3 (3.6)	10.0 (3.9)	10.8 (1.7)
IAP, mm Hg	C-group	4.8 (3.7)	6.0 (2.9)	7.0 (4.0)	7.0 (4.4)	6.8 (4.3)[a]
	B-group	4.2 (2.6)	5.0 (2.4)	5.7 (2.5)	5.5 (2.4)	6.8 (3.7)[a]
PV, ml/kg	C-group	54.6 (8.4)	52.0 (9.6)	51.7 (9.9)	52.0 (10.0)	53.0 (9.6)
	B-group	51.8 (4.7)	46.5 (5.9)	41.7 (3.0)	41.0 (4.0)	42.1 (4.7)[b, *]
FER, ml/kg·min	C-group	0.21 (0.03)	0.26 (0.06)	0.23 (0.04)	0.20 (0.04)	0.20 (0.03)[d]
	B-group	0.21 (0.03)	0.31 (0.07)	0.31 (0.06)[b]	0.21 (0.04)	0.19 (0.04)[d]
Protein mass, g/kg	C-group	2.46 (0.55)	2.28 (0.58)	2.06 (0.45)	2.12 (0.60)	2.02 (0.39)[c]
	B-group	2.41 (0.22)	2.09 (0.27)	1.78 (0.26)	1.69 (0.22)	1.68 (0.18)[b]

Results are mean with SD in parentheses.

MAP = Mean arterial pressure, MPAP = mean pulmonary arterial pressure, CVP_F = femoral venous pressure, PVP = portal venous pressure, IAP = intra-abdominal pressure.

* $p < 0.05$ (between group differences), [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$ (within-group differences as compared with baseline values), [d] $p < 0.01$ (within-group differences as compared with values after 120 min).

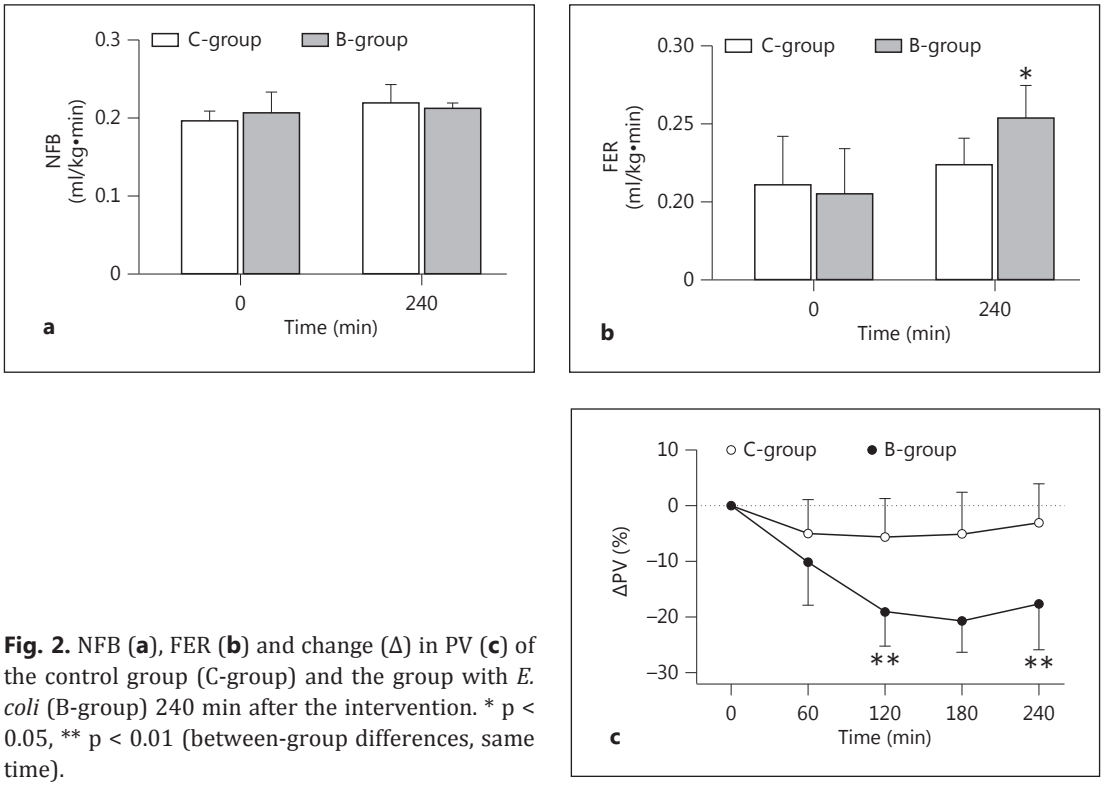

Fig. 2. NFB (**a**), FER (**b**) and change (Δ) in PV (**c**) of the control group (C-group) and the group with *E. coli* (B-group) 240 min after the intervention. * $p < 0.05$, ** $p < 0.01$ (between-group differences, same time).

PV of the C-group was essentially unchanged, with a slight reduction of 3.1 (7.0)% as compared to a 18.6 (7.9)% reduction in the B-group ($p < 0.01$ between-group difference).

The average FER in the C-group was 0.21 (0.03) before and 0.22 (0.02) ml/kg/min after the intervention ($p > 0.05$), while it increased from 0.21 (0.03) to 0.25 (0.02) ml/kg/min in the B-group ($p < 0.05$ between-group difference). Protein masses of both study groups were reduced, but the values of the B-group tended to be lower than those of the C-group after 240 min (table 1).

Biomarkers are presented in figure 3. TNF-α in the blood samples from the femoral artery was stable in the C-group with baseline values of 102.1 (56.9) and 68.3 (18.0) pg/ml after 240 min. The corresponding values of the B-group increased from 109.3 (38.7) to 601.7 (519.7) pg/ml with a maximum value of 1,310.7 (1,352.4) after 120 min. Significant differences between the groups were seen after 120 and 240 min ($p < 0.01$). In the portal vein of the C-group, TNF-α also remained low with initial values of 104.6 (63.3) and 74.6 (19.8) pg/ml at the end of the experiment. In the B-group, the TNF-α values from the portal vein were 98.3 (34.5) and 612.0 (582.4) pg/ml. When comparing the TNF-α values from the systemic and portal circulation of the B-group, the levels were almost identical in the two groups at all corresponding time points (fig. 3).

A similar pattern was observed in the levels of IL-6. The values of the C-group were 372.3 (266.5) and 231.9 (174.2) pg/ml in the femoral artery and 426.3 (299.8) and 249.9 (189.2) pg/ml in the portal vein at baseline and after 240 min. The levels of IL-6 in the B-group were 235.7 (257.5) and 2,172.5 (1,283.4) pg/ml in the femoral artery, and 255.7 (241.3) and 2,154.2 (1,130.2) pg/ml in the portal vein at baseline and after 240 min. In the B-group, IL-10 increased from 19.2 (20.7) pg/ml at baseline to a peak of 61.0 (31.5) pg/ml after 120 min in portal blood ($p < 0.01$). A similar trend was seen in the femoral artery, while the values from

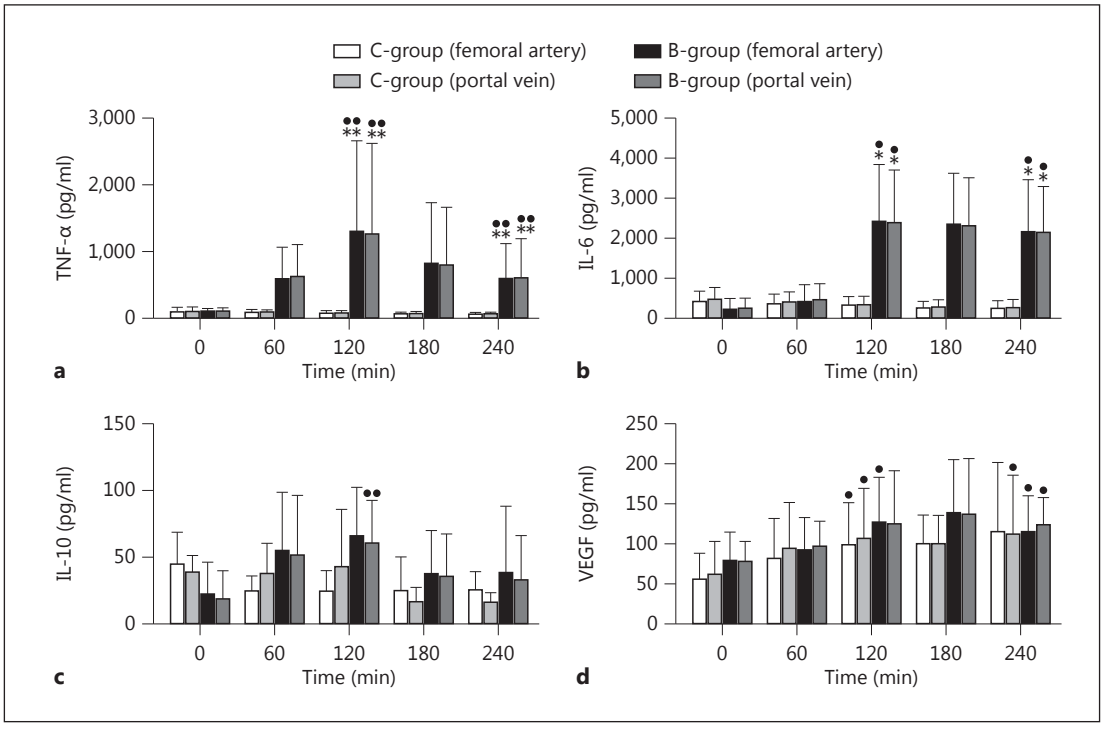

Fig. 3. TNF-α (**a**), IL-6 (**b**), IL-10 (**c**) and VEGF (**d**) in the femoral artery and the portal vein of the control group (C-group) and the group with *E. coli* (B-group) 240 min after the intervention. * $p < 0.05$, ** $p < 0.01$ (between-group differences, same time). • $p < 0.05$, •• $p < 0.01$ (within-group differences compared with baseline values).

the C-group remained below 45 pg/ml. No significant between-group difference was found though. VEGF slightly increased in portal and femoral blood from both study groups during the intervention, but the groups did not differ significantly from each other at any time. The results of the biochemical analysis and portal vein microdialysis are displayed in table 2. Hct and haemoglobin increased significantly in the B-group, with a between-group difference at the end of the experiments.

Serum leukocyte count was reduced in the B-group during the experiments and differed from the C-group at the end ($p = 0.05$). The portal level of glucose, measured by microdialysis, was reduced in both study groups during the experiments ($p < 0.05$) as was the level of blood glucose in the C-group ($p < 0.05$).

The total water content of various tissues and organs are presented in table 3. The kidneys of the C-group had a significantly higher water content as compared to the B-group ($p < 0.05$).

Average urinary outputs were 1.01 (0.39) and 0.54 (0.43) ml/kg/min in the C-group and the B-group, respectively ($p < 0.05$). The core temperature increased from 38.4 (1.4) to 39.3 (1.4) °C in the C-group and from 38.0 (1.0) to 39.4 (1.1) °C in the B-group ($p > 0.05$).

Discussion

The present study was performed to assess the portal venous contribution to the systemic level of cytokines and to validate the experimental model of early bacterial peritonitis and sepsis in pigs with respect to clinical relevance.

Table 2. Results of the biochemical analysis and portal vein microdialysis[a]

	Group	Baseline	60 min	120 min	180 min	240 min
Hct	C-group	29.6 (3.4)	30.7 (4.2)	30.6 (4.5)	30.3 (4.3)	29.6 (4.0)
	B-group	31.0 (1.2)	33.1 (1.6)	35.1 (2.0)	35.4 (1.5)	34.7 (2.1)[c, *]
Haemoglobin, g/dl	C-group	9.9 (1.0)	10.0 (1.2)	10.1 (1.2)	9.9 (1.2)	9.6 (1.2)
	B-group	10.4 (0.4)	11.1 (0.4)	11.7 (0.7)	11.7 (0.7)	11.5 (0.9)[b, **]
Leukocytes, 10^9/l	C-group	22.6 (8.0)	23.7 (11.0)	25.8 (13.9)	27.4 (13.0)	28.1 (13.2)
	B-group	21.2 (8.2)	15.4 (4.4)	13.2 (5.2)	13.8 (5.7)	15.0 (6.4)[b]
Serum sodium, mmol/l	C-group	137.3 (1.1)	136.0 (1.6)	135.1 (1.8)	135.3 (1.5)	135.1 (1.8)[c]
	B-group	139.0 (1.7)	138.0 (1.3)	138.0 (1.9)	137.2 (1.0)	136.3 (1.2)[c]
Serum potassium, mmol/l	C-group	4.7 (0.5)	5.1 (0.6)	5.8 (1.0)	6.2 (1.1)	6.5 (1.2)[c]
	B-group	4.6 (0.5)	5.2 (0.6)	5.8 (0.5)	6.2 (0.6)	7.0 (0.7)[d]
Serum osmolality, mosm/kg	C-group	284.9 (3.2)	283.0 (3.7)	280.3 (3.4)	279.7 (3.8)	280.3 (2.6)[c, ***]
	B-group	286.7 (1.9)	286.3 (3.7)	286.5 (2.4)	286.5 (2.1)	287.2 (2.0)
Serum glucose, mmol/l	C-group	6.1 (2.3)	5.0 (1.2)	4.3 (1.3)	3.5 (0.8)	3.0 (1.1)[b]
	B-group	5.6 (1.6)	5.1 (1.9)	4.7 (2.2)	4.3 (1.9)	4.1 (2.0)
PaO_2, kPa	C-group	30.5 (5.5)	27.9 (4.6)	27.9 (6.1)	29.1 (3.3)	30.5 (3.5)
	B-group	34.1 (6.7)	33.6 (5.8)	32.4 (4.9)	32.2 (6.9)	30.7 (4.5)
$PaCO_2$, kPa	C-group	5.7 (0.8)	5.3 (0.8)	5.3 (0.9)	5.2 (0.9)	5.1 (1.1)
	B-group	5.2 (0.5)	5.6 (0.8)	5.5 (1.0)	5.2 (0.7)	5.3 (0.6)
pH	C-group	7.47 (0.1)	7.49 (0.04)	7.50 (0.1)	7.50 (0.05)	7.51 (0.1)
	B-group	7.50 (0.1)	7.47 (0.1)	7.46 (0.1)	7.49 (0.1)	7.48 (0.1)
Base excess, mmol/l	C-group	6.1 (1.4)	5.3 (1.9)	6.2 (1.2)	6.2 (1.5)	6.1 (1.5)
	B-group	6.4 (2.4)	5.6 (1.8)	4.3 (2.1)	4.8 (1.2)	4.9 (2.3)
MD glucose[a], mmol/l	C-group	5.3 (1.5)	4.6 (1.5)	4.1 (1.8)	4.0 (1.8)	3.4 (0.5)[b]
	B-group	6.1 (1.3)	5.8 (1.3)	5.3 (1.2)	4.3 (2.1)	3.6 (1.8)[b]
MD lactate[a], mmol/l	C-group	1.5 (1.1)	2.0 (1.4)	1.9 (1.2)	1.6 (1.1)	1.6 (1.3)
	B-group	2.1 (0.9)	1.7 (0.6)	2.1 (1.4)	2.0 (0.9)	2.8 (2.0)
MD/LP ratio[a]	C-group	12.4 (5.1)	16.6 (8.5)	15.4 (6.5)	13.9 (9.3)	16.6 (9.9)
	B-group	19.5 (6.9)	18.9 (6.0)	18.4 (5.9)	18.7 (4.2)	22.9 (9.5)

Results are mean with SD in parentheses.

MD/LP = Lactate/pyruvate.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (between-group differences). [a] Portal vein microdialysis. [b] $p < 0.05$, [c] $p < 0.01$, [d] $p < 0.001$ (within-group differences as compared with baseline values).

We found elevated values of TNF-α and IL-6 in the portal and systemic circulation, and the levels were nearly identical with respect to both the temporal development and the magnitude of the response. Even though there was an increase in IL-10 in portal blood from the B-group animals after 120 min, no significant difference was obtained between the study groups. Accumulating evidence supports the idea of a mixed pro- and anti-inflammatory response in the early stages of sepsis. Human studies on postoperative sepsis and septic shock have revealed a parallel increase in pro- and anti-inflammatory cytokines from the first day of disease, and murine studies have shown an increase in IL-10 after 3 h of septic peritonitis [9, 10]. The explanation for the modest IL-10 response in the present study is not clear but could be related to the short duration of each experiment or the limited number of animals in the study groups. VEGF is produced by macrophages and lymphocytes in severe sepsis and polytrauma and has the capability to cause vasodilatation and increased capillary permeability [11]. The present study was not able to demonstrate between-group differences in VEGF, which is possibly due to the limited observational period.

It is well recognised that bacterial peritonitis and sepsis are accompanied by elevated levels of circulating pro-inflammatory cytokines. The contribution from circulating leuco-

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Table 3. Total tissue water content (g/g dry weight)

	C-Group	B-group
Right heart	4.30 (0.14)	4.10 (0.29)
Left heart	4.09 (0.12)	4.09 (0.06)
Lung	4.90 (0.49)	4.46 (0.37)
Liver	4.21 (0.29)	3.90 (0.57)
Right kidney	5.49 (0.23)	5.10 (0.37)*
Left kidney	5.35 (0.21)	4.94 (0.30)*
Ventricular mucosa	5.18 (0.59)	5.33 (1.25)
Ventricular muscularis	5.12 (0.79)	5.06 (0.38)
Pancreas	3.74 (0.55)	3.59 (0.38)
Ileum muscularis	4.50 (0.53)	4.56 (0.63)
Ileum mucosa	5.73 (0.42)	5.68 (0.29)
Colon	4.58 (1.49)	4.91 (0.84)
Peritoneum	2.56 (1.05)	2.53 (1.82)
Omentum	1.03 (0.26)	0.81 (0.30)
Skin	2.86 (0.40)	2.48 (0.35)
Striated muscle	3.83 (0.32)	3.62 (0.20)
Brain	4.56 (0.40)	4.45 (0.27)

Results are mean with SD in parentheses.
* $p < 0.05$ (between-group differences).

cytes is normally limited to the early phase [12, 13]. The gut is capable of producing inflammatory cytokines, but the contribution to the systemic levels of cytokines is unclear. In the present study, no gradient was seen between the levels of the portal vein and the femoral artery, indicating that that the contribution from the intestine, spleen and mesenterial macrophages were of minor significance. Similar results were reported in a rat model of peritonitis by caecal ligation and puncture when comparing the levels of TNF-α, IL-1β and IL-6 in the portal and systemic circulation [14]. Even when chemical peritonitis was induced in rats by zymosan, the levels of TNFα and IL-6 were similar in the portal and systemic circulation [15]. However, a study on experimental peritonitis and abscess formation in rats showed a 10% higher concentration of IL-6 in the portal vein as compared to the inferior vena cava [16]. The observational period in this study was 3 days and hence the results are not fully comparable with the present study.

The efferent mesenteric lymphatic vessels could be a route of cytokine transport from the abdominal cavity to the systemic circulation. Indeed, elevated values of IL-6 in mesenteric lymphatic fluid have been demonstrated in zymosan-induced peritonitis in rats. The thoracic duct of patients with multiple organ failure has been shown to contain elevated levels of TNF-α and IL-6 as compared to the systemic values [17, 18]. The importance of the lymphatic route, however, is unclear. Available data support the concept that some factors in the mesenteric lymphatic fluid are responsible for triggering a systemic inflammatory response in trauma/hemorrhagic shock or sepsis. The exact nature of these factors is unknown, however. According to current evidence, the responsible factors are probably neither endotoxin nor cytokines [19, 20].

The Kupffer cells of the liver constitute 80–90% of the macrophages present in the body and have a large capacity for producing cytokines when exposed to endotoxin, bacteriae or inflammatory mediators from the portal vein [21]. Previous studies on experimental bacterial peritonitis have shown that the liver is exposed for endotoxin by the portal vein [22]. Thus, the liver may be an essential source of systemic pro-inflammatory mediators during perito-

nitis. A reduced number of Kupffer cells has been associated with a lack of IL-6 up-regulation in rats with peritonitis after caecal ligation and puncture [23].

The physiologic response to peritonitis in the present study was similar to the changes seen in human sepsis and characterised by enhanced capillary fluid filtration, PV contraction and reduced urinary output. The B-group also underwent a significant reduction in leucocyte count. The usual hyperdynamic circulation was absent, however. This observation could be partly explained by hypovolemia since the clinical routine of fluid resuscitation during sepsis was not applied in this model. Another contributing factor could be septic myocardial depression and elevated pulmonary vascular resistance as observed in the B-group, leading to increased right ventricular afterload. The latter phenomenon has frequently been observed in experimental but also in human sepsis, and has been ascribed to constriction of the pulmonary veins mediated by endothelin [24]. The levels of glucose and lactate as well as the lactate/pyruvate ratio in the portal vein were stable and did not differ between the study groups. Despite the diminished cardiac index, the intestinal supply of energy substrates and oxygen appeared to be adequate in both study groups within the observation period. Urinary output was higher in the C-group, and the kidney water content was elevated as compared with the B-group. The explanation is not clear but could be related to the higher water content in the renal tubule system. The kidney capsule normally limits oedema formation, and therefore interstitial fluid accumulation appears to be less likely.

The current experimental model is based on a mono-microbial infection and has features in common with spontaneous bacterial peritonitis as well as with peritonitis associated with peritoneal dialysis treatment. Similar models have been criticised for not providing bacterial growth in vivo and therefore rather being a model of acute endotoxin release [25, 26]. However, given the intra-peritoneal localisation of endotoxin, the model should still be suitable for studies on the mechanism of how the inflammatory process is spread to the systemic compartment. Furthermore, the use of a porcine model for studies on peritonitis is supported by the close relationship between macrophage response in pigs and humans as opposed to the response in murine models [27].

We conclude that the contribution of TNF-α and IL-6 from the portal vein to the systemic circulation during acute bacterial peritonitis is of minor importance in this experimental porcine model. The present experimental model has essential features in common with early stages of human bacterial peritonitis and may be used in studies on the mechanisms triggering systemic inflammatory response.

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Disclosure Statement

No conflicts of interest exist for any of the authors.

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