Journal of Surgical Research **134,** 133–137 (2006)
doi:10.1016/j.jss.2005.12.007

Portal and Systemic Endotoxemia in Abdominal Operations: The Significance of Acute Abdomen

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Submitted for publication September 2, 2005

Little evidence is available for the implication of bacterial translocation in cases of acute abdomen. Intraoperative endotoxemia in both portal and systemic circulation was studied in 20 surgical patients with acute abdomen and in 36 controls undergoing elective abdominal surgery. Blood was sampled simultaneously from a mesenteric vein immediately after opening the peritoneum and from a peripheral vein. Endotoxin was measured by a colorimetric Limulus amebocyte lysate assay and malondialdehyde (MDA) was measured by the thiobarbiturate assay and passage through a high-performance liquid chromatography (HPLC) system as a marker of the oxidative status. LPS concentrations (mean ± SE) in portal vein blood from patients with acute abdomen was 5.69 ± 1.58 and from patients with chronic diseases 1.05 ± 0.07 EU/ml ($P < 0.0001$). Respective values for the systemic circulation were 4.98 ± 1.47 and 1.36 ± 0.31 EU/ml ($P < 0.0001$). Concentrations of MDA (mean ± SE) in portal vein blood from patients with acute abdomen was 11.16 ± 4.00 and from patients with chronic diseases was 10.56 ± 2.39 μM (P NS). Positive correlations were observed between endotoxin and MDA in both portal and systemic circulation. These results indicate increased levels of endotoxin in acute abdominal conditions pointing to the gut as the site of origin of the bacterial products.

Key Words: **endotoxemia; bacterial translocation; portal.**

INTRODUCTION

A leading hypothesis in the pathogenesis of sepsis and multiple organ failure (MOF) implicates the gut as the motor of systemic inflammation. Bacteria, bacterial products, and cytokines released from the gut into the systemic circulation are considered the "second hit" in the vicious circle leading to multiple organ failure and death [1].

The entry of bacteria or endotoxin from the gut into the systemic circulation, known as bacterial translocation, has been implicated in various disease conditions to explain the observed bacteremia, including trauma [2] and hemorrhage [3]. In animal models of hemorrhagic shock bacteria have been isolated from the mesenteric lymph nodes and endotoxemia attributed to increased intestinal permeability has been described in systemic circulation [3–5]. In humans the presence of bacteria and endotoxin has been observed in the portal circulation in aortic aneurysm repair surgery [6]; in patients undergoing colectomy for colon cancer bacteria have been isolated from mesenteric lymph nodes [7]. Bacterial translocation has been hypothesized to correlate with increased rate of infectious complications [8].

The gut origin of the endotoxemia can only be confirmed if endotoxins are measured simultaneously at the portal and systemic circulation. Further evidence corroborating this hypothesis would be an increased incidence of endotoxemia in acute conditions compared to elective operations. It could be hypothesized that acute abdomen would be related to higher rates of bacteremia or endotoxemia because of frequent coexistence of gut inflammation or systemic hemodynamic instability. The present study investigated the release of lipopolysaccharide (LPS) into both the portal and the systemic circulation during emergency surgery for acute abdominal conditions compared to that during elective surgery and the correlation to the release of

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0022-4804/06 $32.00

TABLE 1

Demographic Data of Patients Enrolled in the Study

	Emergency surgery	Elective surgery	
Number of patients	20	36	
Male/Female (no.)	17/3	26/10	$P < 0.05$
Age (mean, range)	55.1 (17–82)	65.5 (18–83)	$P < 0.05$
APACHE II score (±SD)	7.52 ± 2.93	7.82 ± 2.78	P NS
Preoperative white blood cell count ($\times 10^3/\mu$l ± SD)	10.44 ± 2.34	10.46 ± 1.88	P NS
Underlying disease (No. of patients)			
Acute cholecystitis	9		
Acute appendicitis	3		
Colon obstruction	5		
Peritonitis	1		
Small bowel obstruction	1		
Splenic rupture	1		
Gallstones		23	
Colon cancer		11	
Hypersplenism		1	
Aortic aneurysm		1	
Operation			
Gastrenteroanastomosis	1	1	
Cholecystectomy	8	24	
Splenectomy	1	1	
Peritoneal exploration	1	1	
Aortic aneurysm repair		1	
Colectomy	6	8	
Appendecectomy	3		

malondialdehyde (MDA) as a marker of intestinal injury and systemic oxidative status.

PATIENTS AND METHODS

Fifty-six patients were enrolled in a prospective study between January 2004 and April 2004 at the Laiko General Hospital in Athens, Greece. Twenty patients were operated due to acute abdominal conditions and 36 underwent elective abdominal operations. Patients with intraabdominal abscess were excluded from the study. Preoperative APACHE II scores, white blood cell counts (WBC) preoperatively, and postoperative complications at 28 days were recorded. The demographic data of the patients are shown in Table 1. All patients received perioperative antimicrobial prophylaxis according to standard protocols with either a second-generation cephalosporin or a beta-lactam/beta-lactamase inhibitor combination. A blood sample was drawn simultaneously from a branch of the mesenteric vein immediately after opening the peritoneum and from a peripheral vein. Blood samples for endotoxin measurement were collected in endotoxin-free tubes and centrifuged (4000 *g* for 10 min) and serum was stored at −70°C in endotoxin-free aliquots until assayed. Blood was also inoculated on McConkey agar plates and incubated for 24 h. Isolate identification was carried out using routine laboratory methods.

LPS was measured in serum by the colorimetric Limulus Amebocyte Lysate assay (QCL-1000 LAL assay, BioWhitaker, MD) according to the manufacturer's instructions and expressed in endotoxin units per milliliter (EU/ml). All measurements were carried out in duplicate and the lower limit of detection was 0.1 EU/ml. Serum was diluted 1:10 with pyrogen-free water and heated at 70°C for 5 min for inactivation of serum factors that inhibit the LAL assay. The endotoxin standard curve was established by serial dilutions of the lyophilized endotoxin standard by *Escherichia coli* O55:B5. Aliquots of 50 μl were incubated for 10 min with 50 μL of enzyme at 37°C. Then 100 μl of a chromogenic substrate solution was added to the wells. The reaction was stopped after 6 min by the addition of 10% acetic acid; absorption was measured at 405 nm.

Lipid peroxidation was estimated by the concentration of MDA. A 0.1-ml aliquot of each sample was mixed to 0.9 ml trichloroacetic acid 20% (Merck, Rahway, NJ) and centrifuged at 12,000*g* and 4°C for 10 min. The supernatant was removed and incubated with 2 ml thiobarbituric acid 0.2% (Merck) for 60 min at 90°C. After centrifugation, a volume of 10 μl of the supernatant was injected into a high-performance liquid chromatography system (HPLC, Agilent 1100 Series, Waldbronn, Germany) with the following characteristics of elution: Zorbax Eclipse XDB-C18 (4.6 × 150 mm, 5 μm) column (Agilent) under 37°C; mobile phase consisting by a 50 mM K_3PO_4 (pH 6.8) buffer and methanol 99% at a 60/40 ratio with a flow rate of 1 ml/min; fluorometric detection with signals of excitation at 515 nm and emission at 535 nm. The retention time of MDA was 3.5 min and it was estimated as μmol/L by a standard curve created with 1,1,3,3-tetramethoxy-propane (Merck). All determinations were performed in duplicate.

Statistical analysis. Concentrations were expressed as means (± SE). Values were compared between patients undergoing emergency operations for acute surgical conditions and those undergoing elective operations with the use of Mann–Whitney *t*-test. Correlation between portal and peripheral endotoxin and MDA levels was tested by Spearman's rank of order (r_s). Multivariate analysis using linear regression was performed to control for confounding factors, including age, sex, APACHE II score, underlying disease, and type of operation. Differences with values of $P < 0.05$ were considered statistically significant.

RESULTS

LPS concentrations (mean ± SE) in portal vein from patients with acute abdomen was 5.7 ± 1.6 and from patients with chronic diseases was 1.0 ± 0.1 EU/ml ($P < 0.0001$) (Fig. 1). Respective values for the systemic circulation were 5.0 ± 1.5 and 1.4 ± 0.3 EU/ml ($P < 0.0001$) (Fig. 2). There was a positive correlation be-

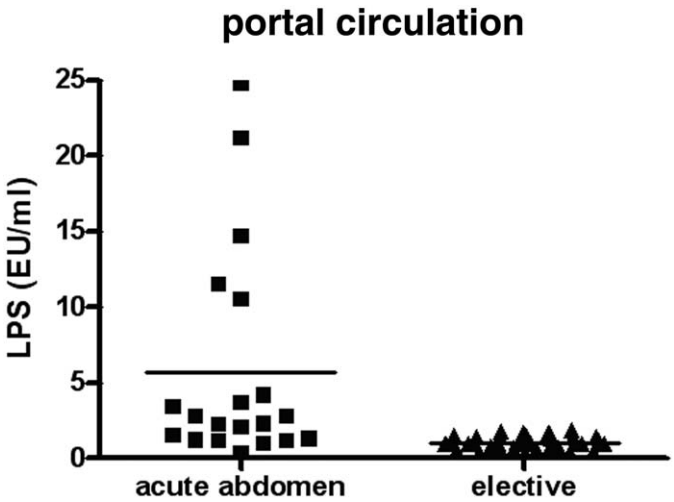

FIG. 1. LPS concentrations in portal blood from patients undergoing emergency surgery for acute abdomen or elective operation ($P < 0.0001$).