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Bacterial Translocation after Partial Hepatic Resection under Ischemia and Reperfusion in Rats: Incidence and Time Course*

Abstract: Infectious complications are major causes of morbidity and mortality following major hepatic resection. We investigated in rats the incidence and time course of bacterial translocation after partial hepatectomy during ischemia and reperfusion. Forty-eight male Wistar rats weighing 230 to 280 g were studied. Partial liver warm ischemia was performed for 15 min followed by reperfusion for 15 min. During a second 15 min of ischemia, 70% hepatectomy was performed. The animals were divided into six groups of eight animals each. Animals were sacrificed 8, 16, 24, 48, 72, and 120 h after the hepatectomy. At each time point blood was drawn for culture, tumor necrosis factor-alpha (TNF-α) and endotoxin determination. Specimens from the mesenteric lymph nodes (MLNs), spleen and liver were harvested for Gram-positive, Gram-negative, and anaerobic bacteria cultures. Plasma endotoxin levels increased significantly in all animals, peaking at 24 h after resection. TNF-α levels increased immediately after surgery, reaching a peak level at 48 h. The incidence of bacterial translocation and the number of translocated bacteria reached their highest levels 48 h after resection. The rate of translocation to both portal and arterial blood was 88% at 48 h and 63% at 24 h. Translocation to intraabdominal organs (liver, spleen and MLNs) was 88% at 48 h and 75% at 72 h. *Proteus* species was cultured most frequently (29%), followed by *Escherichia coli* (23%). In conclusion, bacterial translocation in rats with 70% hepatectomy under ischemia/reperfusion was significantly increased 48 h after surgery. This outcome correlates with serum TNF-α levels.

Key Words: liver ischemia, major hepatectomy, bacterial translocation, endotoxin, TNF-α

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Introduction

Despite developments in modern surgical techniques and intensive care, infectious complications remain important causes of mortality after emergent and elective liver resections (1,2). The reticuloendothelial system (RES) serves as a filter for circulating bacteria and has a vital role in countering any invasion of microorganisms. The liver is an important component of the RES. The increased rates of bacterial translocation during acute liver failure are ascribed to Kupffer cell damage, defective serum opsonization, complement deficiency and decreased intestinal oxygen extraction (3-5). Still, even in the absence of a clinical picture of acute liver failure, it has been shown that live bacteria pass the intestinal barrier and invade extraintestinal organs after major liver resections (6-9). This observation is explained by the impairment of intestinal barrier function and bacterial overgrowth (8,9). Although bacterial translocation after different rates of liver resection has been described, translocation after liver resection subjected to ischemia-reperfusion, which is a more accurate simulation of the clinical situation, has attracted relatively less attention (10,11). In major liver injuries and elective liver resections, portal triad occlusion is performed to limit blood loss (2,12). The clamping results in warm ischemia whereas declamping induces reperfusion damage. The oxygen radicals released cause the secretion of cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10) (13). Consequently, the systemic and metabolic effects of liver resection as well as the cytokine release caused by ischemia-reperfusion bring about a complex pathophysiological picture. The objective of this study was to investigate the frequency and timing of bacterial translocation after 70% hepatectomy performed during warm ischemia and reperfusion of the liver and to elucidate the relationship between bacterial translocation and the proinflammatory cytokine TNF-α.

Materials and Methods

This study was performed after we obtained the approval of the Ethics Committee of Erciyes University. All animals were cared for according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH). Forty-eight male Wistar rats weighing 230-280 g were used. The rats were fasted overnight before the experiments, but were given free access to water. The weights were measured shortly prior to the experiments. Anesthesia with spontaneous breathing was induced by intraperitoneal injection of sodium pentobarbital (50 mg/kg). After shaving the abdominal and neck hair, a polyethylene catheter (internal diameter: 1.1 mm) was placed under sterile conditions into the left internal jugular vein and 200 IU/kg heparin was given. Then laparotomy was performed with a midline incision. Portal and arterial blood to the left lateral and median lobes was interrupted for 15 min with a vascular clip (Harvard Apparatus, Inc. Hollinston, MA, USA). At the end of this period, reperfusion was allowed for 15 min. Then the left lateral and the median lobes were resected (70% hepatectomy) during a second 15-min ischemic period. After removal of the clamps, the abdomen was closed with 3/0 polyglactin-910 sutures. Eight rats were sacrificed at each of the following time points: 8 h, 16 h, 24 h, 48 h, 3 days and 5 days. From each animal, 3 ml of blood was drawn for endotoxin and TNF-α measurements: sera were separated and stored at –80 $^\circ$C until analysis. Two milliliters of portal and systemic blood and liver, spleen and mesenteric lymph node (MLN) biopsies were obtained for bacteriological examination. In addition, another liver biopsy was obtained for histopathological examination.

The analysis of serum endotoxin was carried out in triplicate and measured using a Limulus Amebocyte Lysate assay kit (Sigma Chemical Company, St. Louis, MO, USA) according to the manufacturer's instructions. Endotoxin results were expressed in endotoxin units per milliliter (EU/ml). Plasma TNF-α level was measured in a 96-well microtiter plate by using a TNF-α test kit (BioSource International, Inc., Camarillo, CA, USA) based on an enzyme-linked immunosorbent assay. All samples were tested in duplicate. The plate was read on a Spectra Image (Tecan Group Ltd., Mannedorf, Switzerland) at 450 nm. The concentration of TNF-α was calculated from a standard curve and expressed in picograms per milliliter (pg/ml).

Liver, spleen and MLN samples were immediately homogenized in saline. Aerobic cultures were performed in a blood and eosin-methylene blue (EMB) agar at 37 $^\circ$C for 24 h. Anaerobic cultures were performed by using an anaerobic blood agar in Gas-Pak jars at 37 $^\circ$C for 24 h. Blood samples were cultured in tryptic soy medium at 37 $^\circ$C for 7 days. Positive cultures were transferred to blood and EMB agar. Colonies were identified with standard bacteriological methods.

The livers were fixed with buffered formaldehyde solution, and stained with hematoxylin and eosin. The extent of parenchymal cell injury, sinusoidal congestion, neutrophil infiltration of, venous congestion and fatty degeneration were evaluated.

Statistical Analysis. Values are expressed as mean ± SD. Statistical analysis was performed using SPSS 10.0 for Windows applying Student's t test for comparisons of serum endoxin and TNF-α. For translocation comparisons the Mann-Whitney U test was used. Values were considered significant if p < 0.05.

Results

The plasma endotoxin level was 47 EU/ml at 8 h and it rose to a maximum level of 362 EU/ml at 24 h. The endotoxin levels at 24 and 48 h were significantly higher in comparison with the levels at 8 and 16 h, and 3 and 5 days (P < 0.001). The postoperative course of plasma endotoxin levels is depicted in Figure 1.

After 70% hepatectomy in the setting of ischemia-reperfusion, TNF-α was detectable in the blood at all time points and a peak level of 44.9 pg/ml was measured after 48 h. The levels at 48 h were significantly higher than the levels at 8 h,16 h, 24 h, 3 and 5 days (P < 0.05). The postoperative course of serum TNF-α levels is depicted in Figure 2.

In order to detect bacterial translocation, which may occur after 70% hepatectomy in the setting of ischemia-reperfusion, aerobic and anaerobic cultures of liver, spleen and MLN biopsies as well as portal and aortic blood were performed. Bacterial translocation reached its maximum level 48 h after resection. At 48 h, the frequencies of bacterial translocation were 100% in the liver, 88% in MLNs, portal and systemic blood and 75%
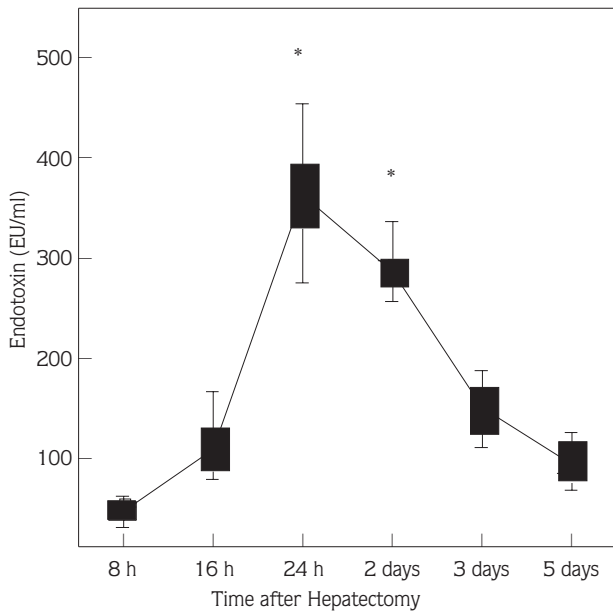
Figure 1.   Effect of hepatic resection under ischemia and reperfusion on plasma endotoxin concentration (* P < 0.001; 24 h and 2 days vs. 8 h, 16 h, 3 days and 5 days).
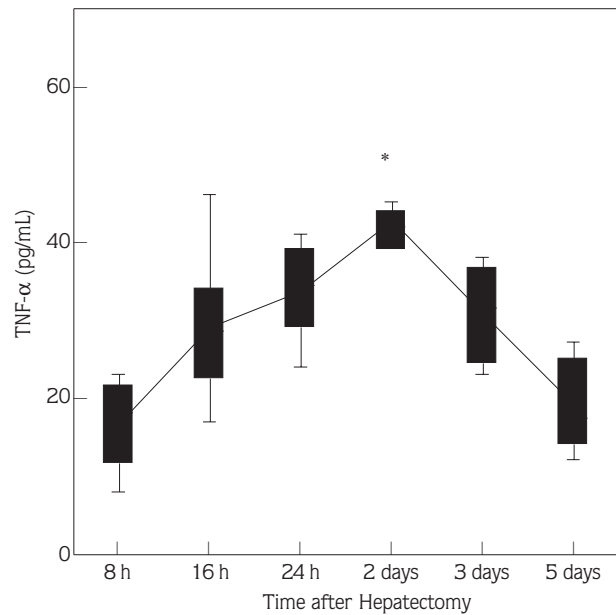

Figure 2.   Serum levels of tumor necrosis factor-α in hepatic resection under ischemia and reperfusion (* P < 0.05; 2 days vs. 8 h, 16 h, 24 h, 3 days and 5 days).

in the spleen. In contrast, in the rats sacrificed 8 h after hepatectomy, bacterial translocation to the liver and spleen were not detected; 38% of the MLN cultures and 25% of the portal and systemic blood cultures were positive for bacteria (Table 1).

Overall, 151 aerobic and five anaerobic species were isolated. The most frequently isolated bacteria were *Proteus* species (29%), *Escherichia coli* (23%),

Staphylococcus aureus (15%) and coagulase-negative *Staphylococci* (13%). The anaerobic bacteria included three Gram-negative *bacilli*, one Gram-positive *bacillus* and one Gram-positive *coccus*. Three of the anaerobic bacteria were isolated from rats sacrificed 24 h after hepatectomy (Table 2).

Histopathological examination of the liver biopsies showed that parenchymal damage was maximum 24 h

Table 1. Frequency and time course of bacterial translocation.

	8 h	16 h	24 h	2 days	3 days	5 days
Liver	-	3	5*	8*,**	6*	2****
Spleen	-	4*	5*	6*	5*	3
MLNs	3	5	5	7*	7*	3****,*****
Portal blood	2	2	5	7*,**	4	1***,****
Systemic blood	2	3	5	7*,**	3****	4

Number of rats with positive bacterial cultures: 8 animals per group

    *   P < 0.05 (8 h vs. 16 h, 24 h, 2 days, 3 days)
   **   P < 0.05 (16 h vs. 2 days)
  *** P < 0.05 (24 h vs. 5 days)
 **** P < 0.05 (2 days vs. 3 days and 5 days)
***** P < 0.05 (3 days vs. 5 days)

Table 2. Spectrum of enteric bacterial translocation.

	N	
Aerobic	151	
Proteus species		45
Escherichia coli		36
Staphylococcus aureus		23
Cogulase-negative *staphylococcus*		21
Klebsiella species		8
Diptheroids		5
Enterococcus		4
Others		9
Anaerobic	5	
Gram-negative *bacilli*		3
Gram-positive *bacillus*		1
Gram-positive *coccus*		1

after hepatectomy. On the other hand, sinusoidal congestion and neutrophil infiltration were at maximum levels 8 h after hepatectomy. Venous congestion was evaluated as mild in all rats. Fatty degeneration in the remnant liver reached maxima after 3 and 5 days.

Discussion

Bacteria and endotoxins can pass through the gut under certain conditions. This process of bacterial translocation may occur after major liver resection and is thought to play a role in the pathogenesis of septic complications. The pathophysiologic mechanisms by which bacterial infection or bacteremia occur in patients subjected to major liver resection are well known. Physical disruption of the intestinal mucosal barrier, bacterial overgrowth and decreased capacity of hepatic RES are major factors in the development of bacterial translocation after hepatectomy (3,8,14). Wang et al. have reported that the incidences of intra-abdominal sepsis and bacteremia caused by enteric bacteria in patients and bacterial translocation in rats are proportional to the extent of liver resected (9). Clinically, hepatic resection is usually performed with total or partial occlusion of blood inflow to the liver to minimize intraoperative blood loss (2,12). There are two aspects of ischemia and reperfusion injury of the liver. One is the injury caused by ischemia and portal congestion during the interruption of hepatic blood inflow, and the other is the reperfusion injury caused by pooled portal blood in

the ischemic liver (15). However, the effect of temporary arterial and portal occlusion on bacterial translocation after hepatectomy remains unclear. Ferri suggested that a significant bacterial translocation in the MLNs occurs after portal triad clamping and liver resection without clinical relevance (10). In the present study, we examined the frequency and timing of bacterial translocation due to hepatectomy under partial ischemia-reperfusion. When compared with bacterial translocation rates after hepatectomy without ischemia/reperfusion in previous studies (9,16,17), our results indicate that the bacterial translocation rates after hepatectomy under ischemia were lower. For example, Wang et al. found that the frequency of bacterial translocation to the MLN was 83% at 24 h after 70% hepatectomy (9). However, we found bacterial translocation to MLNs was 63% after 24 h. In another study, Wang et al. found that the incidence of bacterial translocation to the systemic circulation, MLN and liver were 30, 80 and 50% respectively, at 12 h after 70% hepatectomy (17). In our study, the rate of bacterial translocation to the systemic circulation, MLN and liver was 25, 38 and 0% respectively at 8 h and 38, 63 and 38% respectively at 16 h. We could not explain which mechanism was exactly responsible for the lower rates of bacterial translocation. However, partial ischemia was induced for 15 min, followed by 15 min of reperfusion in our study. Thereafter partial ischemia was induced again for 15 min. To our knowledge, this is a protocol typical for ischemic preconditioning. We think this could be related in some way to the difference in bacterial translocation.

The risk of bacterial invasion is increased with time after hepatectomy and the ability to clear bacteria decreased immediately following major hepatectomy. Enteric bacteria translocate from the intestine between 2 and 12 h after liver resection and the clearance of these invasive bacteria starts 2 to 4 days after hepatectomy (8-10,14). Wang et al. have shown that in the setting of postoperative liver failure due to 90% hepatectomy, bacterial translocation starts 2 hafter surgery and is associated with the overgrowth of *E. coli* in the intestines (4). However, infectious complications may develop days and even weeks after liver resection (18,19). Kasravi et al. reported that after 70% hepatectomy, the frequencies of translocation to the portal and systemic blood were 100% at 24 h and 50% at 48 h (20). However, the frequencies for the liver, spleen and MLNs were 100% at

both time points. In the present study, in order to evaluate long-term results, we investigated bacterial translocation between 8 h and 5 days after hepatectomy. We found that bacterial translocation was most intense 48 h. after hepatectomy. The spectrum of the bacteria translocated after liver resection mirror the intestinal flora such as *E. coli, Proteus* species, enterococcus, and *Klebsiella* (4,8). In our study, *Proteus* species were most common (29%) and these were followed by *E. coli* (23%).

The interruption of hepatic inflow induces warm ischemia and reperfusion injury resulting in impaired postoperative liver function (21,22). On the other hand, the effective control of intraoperative bleeding is one of the most important measures for successful hepatectomy. Liver ischemia results in TNF-α and IL-10 production and the peak levels of these cytokines are directly related to the duration of cold ischemia (13,23,24). However, ischemia alone is inadequate and reperfusion should also take place. Recent studies show that increased oxygen metabolites play a role in TNF-α production in vivo (25). Le Moine et al. have shown that TNF-α and IL-10 released from human monocytes are stimulated by hydrogen peroxide (24). The liver is known to be an important site of TNF-α and IL-6 production during infection, but the interactions between these cytokines, surgical injury and perioperative infection are poorly understood in patients undergoing liver resection. The clearance of bacteria and bacterial products from portal blood is an important function of Kupffer cells. If the capacity of the Kupffer cells is overwhelmed after hepatectomy, endotoxin spills out from the Kupffer cells

and directly injures the hepatocytes (26). TNF-α is responsible for nearly all of the effects ascribed to endotoxins. IL-6, also a product of lipopolysaccharide-activated Kupffer cells, may be instrumental in eliciting the acute-phase response of hepatocytes. Although Yachida (19) and Suzuki (27) showed that TNF was undetectable in the serum 48 h after hepatectomy, Iwai (28) showed that the secretions of TNF and IL-6 from the perfused rat liver were increased after 67% partial hepatectomy, reaching a maximum after 48 h. In our study, endotoxin and TNF-α were detectable in the serum at all time points with endotoxin reaching a peak level at 24 h and TNF-α reaching a peak level at 48 h. Whether the discrepancy is due to the fact that we performed hepatectomy under ischemia-reperfusion warrants further study.

In conclusion, liver resection in the setting of ischemia-reperfusion results in TNF-α and endotoxin release. The timing and frequency of bacterial translocation after liver resection are parallel to the course of TNF-α levels. Further studies are needed to evaluate bacterial translocation and its relationship with cytokines in rats following hepatic resection after ischemic preconditioning.

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