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Selective bowel decontamination improves the survival of 90% hepatectomy in rats

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ABSTRACT

Background: Clinically, hepatectomy is a clean procedure performed without routine antimicrobial prophylaxis, regardless of the extent of liver loss. Translocation of endotoxin has been recognized as a fatal complication leading to liver failure. After extended hepatectomy, the portal hypertension, mucosal damage, intrahepatic bile acid retention, inhibited enterokinesia, and so forth are likely to contribute to enhanced endotoxin absorption. The effect of selective bowel decontamination (SBD) on the prognosis of hepatectomy were investigated.

Methods: We adopted rat models of partial hepatectomy (70%, PHx) and subtotal hepatectomy (90%, SHx), gentamicin or saline of the same amount was administrated preoperatively. Liver damage makers, portal and systemic lipopolysaccharide, mucosal damage, signaling pathways, liver regeneration, and bile canalicular networks reconstruction were investigated.

Results: We found that SHx but not PHx resulted in significantly enhanced portal and systemic endotoxin. Inhibition of gastrointestinal gram-negative bacteria by gentamicin significantly reduced lipopolysaccharide levels and improved survival after SHx (56% with gentamicin, 24% with saline, $P < 0.05$). We also found SBD with gentamicin protected intestinal mucosa barrier, alleviated liver parenchymal damage, and promoted liver regeneration and bile canalicular networks reconstruction after extended liver resection.

Conlusions: We conclude that SBD is beneficial and necessary for extended hepatectomy.

1. Introduction

The gastrointestinal bacterial flora contains a certain amount of gram-negative bacteria that produces lipopolysaccharide (LPS), an endotoxin. The presence of endotoxins in the systemic blood is prevented via several defending mechanisms [1]. The colonization resistance and normal enterokinesia inhibited the overgrowth of the gram-negative bacteria. The anatomic integrity of the normal mucosal barrier and the conjugation of LPS with bile acids into unabsorbable complex suppressed invasion of the bacteria and endotoxin. Then, portal endotoxin is cleared by the Kupffer cells when it reaches the liver. If a surgical procedure involves the gastrointestinal tract, which would necessarily disturb one or more of the defending mechanisms, antimicrobial prophylaxis would be recommended or obligatory [2].

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Hepatectomy is often the only curative option for various kinds of liver diseases [3]. Regardless of the extent of liver loss, antimicrobial prophylaxis is recommended only if the procedure involves bile duct reconstruction or entry into the gastrointestinal tract [2]. Otherwise, the procedure is generally considered clean, which does not require routine antimicrobial prophylaxis or bowel decontamination. However, after liver resection, the temporal portal hypertension and bowel congestion would affect intestinal permeability and enterokinesia [4,5]. The regeneration of the liver would lead to temporal intrahepatic bile acid retention and decrease of bile acid in the gastrointestinal tract [5,6]. These factors would likely lead to bacterial overgrowth and increased portal LPS. In the scenario of extended hepatectomy, the high incidence of postoperative liver dysfunction due to insufficient remnant liver would lead to prolonged cholestasis and coagulopathy, portal hypertension, or even ascites, which, if progresses, predisposes to further complications including sepsis and gastrointestinal bleeding [3]. We hypothesize that liver failure after extended liver resection were to partially be attributed to enhanced endotoxin translocation and tested the effect of selective bowel decontamination (SBD) in improving the prognosis of extended hepatectomy.

The present study investigated the effect of SBD on different extent of liver resection. We adopted rodent models of subtotal hepatectomy (90%, SHx) and partial hepatectomy (70%, PHx). Oral administration of gentamicin was used for SBD. Gentamicin is an aminoglycoside antibiotic used to treat gram-negative bacterial infections. It is not absorbed when given orally and is active only in the gastrointestinal tract. Our results showed that endotoxin and bacterial translocation was significantly enhanced after extended hepatectomy but not after partial hepatectomy, which could be attenuated by SBD, leading to improved survival after extended hepatectomy.

2. Materials and methods

2.1. Animal models

The experiments were performed on 7-wk-old male Sprague–Dawley rats, weighing 220–250 g (purchased from the Laboratory Animal Research Center of the Academy of Military Medical Science). Animal procedures were approved by the Institutional Animal Care and Use Committee. SBD was achieved by intragastric administration of gentamicin (dissolved in saline, 5 mg/mL, dosage 5 mL/kg) at 8 AM for five consecutive days, with saline of the same volume used as control (Fig. 1A), after which six rats with gentamicin or saline administration were sacrificed to evaluate gut bacterial population. All the surgical procedures were performed between 9 AM and 12 AM by an experienced surgeon with sufficient microsurgery training. Ether inhalation was used as anesthesia in all procedures. To evaluate the effect with gentamicin after hepatectomy, rats were randomized into five groups as follows: sham, 90% hepatectomy with saline (subtotal hepatectomy, SHx) or gentamicin administration (G-SHx), and 70%, hepatectomy with saline (partial hepatectomy, PHx) or gentamicin administration (G-PHx). Liver resections were performed as previously reported [7,8]. Rodent liver were lobulated. Briefly, the bile duct, portal vein, hepatic artery, and the hepatic vein to the intended lobes were ligated before transected, and the liver lobes were then removed. In SHx, all liver lobes except the whole caudate lobe were resected. However, in PHx, the middle and the left-lateral lobes were removed. The rats were sacrificed at indicated time points after operation. At least six rats were examined for each time point. In a separate study, 25 rats were used to examine the survival rate in the SHx group and 25 in the G-SHx group, and five in each of the other three groups (Fig. 1A).

2.2. Serum aspartate aminotransferase, alanine aminotransferase, bile acids, and total bilirubin levels

Levels of serum alanine aminotransferase, aspartate aminotransferase, total bile acids, and total bilirubin (T.Bil) were measured using a serum analyzer (Cobas-Mira Plus; Roche, Manheim, Germany).

2.3. Limulus amebocyte lysate assay

Endotoxin was investigated in both portal and systemic blood. The portal blood was taken first, followed by ligation of the portal vein. The systemic blood was then obtained from the inferior vena cava. The blood samples were taken with pyrogen-free heparinized tubes. Plasma LPS levels were quantified by the limulus amebocyte lysate test according to the manufacturer's protocol (QCL-1000; Lonza, Bern, Switzerland). Briefly, 50 μL of the test sample was added to the test well in the 96-well plate at 37°C. At least three wells were used for each sample. The linearity of the standard was verified using LPS supplied in the kit. At $T = 0$, 50 μL of limulus amebocyte lysate was added to each well. At $T = 10$ min, 100 μL of substrate solution was added. At $T = 16$ min, 100 μL of stop reagent was added. After this, absorbance was read at 405–410 nm.

2.4. Bacterial studies

The cecal bacterial population was investigated after the final administration of gentamicin. The cecal contents of the gut were collected by the following technique [9]. The ascending colon was ligated and a needle was introduced in to the cecum through the ileocecal valve with 2 mL of sterile saline infused in the cecum, the needle was withdrawn and the terminal ileum was ligated. The cecal content was massaged for 2 min, then 1 mL was collected by puncturing the cecal wall. The samples were diluted serially in a thioglycollate broth and were inoculated onto 5% blood and Eosin Methylene Blue agar and incubated at 37°C for the growth and identification of bacteria. The populations of gram-negative bacteria was counted and calculated as colony forming unit per milliliter.

2.5. Histology and immunohistochemical staining

The liver and segments of the distal ileum were fixed in a 10% formaldehyde-phosphate-buffered saline solution. For