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Portal Triad Occlusion Induces Endotoxin Tolerance: Role of Portal Congestion[1]

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Background. **Portal triad occlusion (PTO) causes portal congestion and damages the intestinal mucosa, which is associated with portal endotoxemia. However, administration of a sublethal dose of endotoxin results in resistance to its toxic activities. We tested the hypothesis that portal congestion due to PTO induces endotoxin tolerance.**

Materials and methods. **Rats were subjected to PTO for 15 min. In Group 1, male rats underwent laparotomy and, 48 h after the surgery, a lethal dose of *Escherichia coli* lipopolysaccharide was administered. In Group 2, rats were subjected to PTO for 15 min. Then a lethal dose of LPS was administered 48 h after surgery. Group 3 was treated the same as Group 2, except that PTO was performed with portosystemic shunt. Group 4 was also treated same as Group 2, except that rats received polymixin B and neomycin by gavage to eliminate intestinal luminal bacteria before PTO. Survival was examined after the administration of a lethal dose of LPS. Changes in plasma levels of cytokine are also measured after the administration of LPS. The portal endotoxin level in each group after PTO was measured.**

Results. **On survival test, only rats in Group 2 and Group 4 showed significantly higher survival rates. The portal endotoxin level was significantly elevated only in Group 2. The elevation of plasma cytokine levels (IL-6, TNF-α) and NO production (NO_2^-/NO_3^-) in Groups 2 and 4 were inhibited compare to those in Groups 1 and 3.**

Conclusions. **PTO induced LPS tolerance possibly due to portal congestion and subsequent visceral congestion.**

Key Words: **entotoxin tolerance; portal congestion; intestine; nitric oxide; lipopolysaccharide; portosystemic shunt.**

INTRODUCTION

Portal triad occlusion (PTO), which is called the Pringle maneuver, is temporally performed during hepatectomy to minimize hepatic bleeding [1]. Application of this procedure is even advocated during liver surgery [2]. However, PTO can potentially damage the liver and other organs [3, 4]. At the end of hepatectomy, edematous changes in the small intestine or mesentery is sometimes observed. Previous studies demonstrated that PTO increased intestinal permeability [5]. Filos *et al.* demonstrated that PTO caused bacterial translocation and endotoxemia in a rat model [6]. Taken together, PTO may be associated with endogenous endotoxemia due to intestinal mucosal barrier dysfunction. However, sublethal dose of endotoxin exposure may render animals resistant to the second exposure to a lethal dose of endotoxin. Such phenomenon was described as endotoxin tolerance. Therefore, PTO-induced endogenous endotoxemia may facilitate the acquisition of endotoxin tolerance.

This study assessed endotoxin tolerance in animals following PTO and evaluated the influence of portal congestion during the PTO.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee of Hamamatsu University School of Medicine and followed the National Institute of Health guidelines for the treatment of laboratory animals.

Male Sprague Dawley rats weighing 275–300 g (Japan SLC, Inc.

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Hamamatsu, Japan) were fasted for 12 h prior to the experiment with free access to food and water. Surgical procedures were performed under general anesthesia with intraperitoneal injection of sodium pentobarbital (50 mg/kg). The animals were kept on a temperature control board (38 ± 1°C) and allowed to breathe spontaneously.

The animals were randomly divided into four groups prior to the experiment. In Group 1, the abdomen was opened with a midline incision for 15 min, and then the abdominal wall was closed. Forty-eight hours postoperatively, the animals were administered a lethal dose of lipopolysaccharide (LPS) (LPS 0111:B4, 10 mg/kg, ip; Sigma, St. Louis, MO) intraperitoneally. In Group 2, the abdomen was opened the same as in Group 1. Then, PTO was performed by clamping the hepatoduodenal ligament for 15 min. Thereafter, the clamp was removed and the abdominal wall was closed. Forty-eight hours postoperatively, the animals were administered a lethal dose of LPS as in Group 1. In Group 3, to avoid portal congestion by PTO, a portosystemic shunt was established as previously reported [7–9]. Briefly, one end of a polyethylene tube (PE 90; inner diameter, 0.86 mm) was introduced into the superior vena cava via the jugular vein. Subsequently, a cecal branch of the mesenteric vein was exposed. The mesenteric vein was clamped temporally to obtain maximal dilation. The other end of the PE tube was inserted into the dilated portion of the cecal vein. The tube was filled with heparinized saline solution. Then PTO was performed for 15 min. After removal of the clamp, the subsequent course was the same as that in Groups 1 and 2. In Group 4, to prevent growth of intestinal bacteria and eliminate luminal endotoxins, the animals were treated with polymixin B (150 mg/kg/day, Sigma) and neomycin (450 mg/kg/day, Sigma) for 4 days prior to the experiment. They were fed sterilized chow and water, and animals were changed to a freshly sterilized cages everyday. After pretreatment, the abdomen was opened and PTO was performed in the same manner in Group 2. The lethal dose of LPS was given 48 h postoperatively. Survival test or blood sampling was subsequently performed in each group. Throughout the experiments, LPS with a same lot number was used.

Survival Rate

Survival rates were compared among Group 1, Group 2, Group 3, and Group 4 ($n = 10$ in each group). After treatment with a lethal dose of LPS, the animals were returned to their individual cages.

Measurement of Portal Endotoxin Level

Portal venous blood samples were centrifuged at 3000 rpm for 5 min, and plasma was stored at −80°C until analysis. In Group 1, the blood samples were taken from the portal vein in the pyrogen-free tube after laparotomy. In Groups 2, 3, and 4, the blood samples were taken from the portal vein immediately after removing the clamp on the hepatoduodenal ligament. The level of endotoxin in each sample ($n = 10$) was measured by the endospecy test (Seikagaku Kogyo, Tokyo, Japan), a chromogenic assay based on limulus coagulation enzymes [10]. This test is specific for bacterial endotoxins because there is no reaction to β-glucan and the detection limit is 0.2 pg/mL.

Measurement of Plasma Cytokine and NO_2^-/NO_3^- Levels

Blood samples were taken from the right jugular vein ($n = 5$), at 0, 3, 6, and 9 h after intraperitoneal administration of LPS. These samples were centrifuged at 5000 rpm for 10 min. Plasma levels of TNF-α and IL-6 were measured with an immunoassay kit (raTNF-α kit, IL-6 kit; BioSource International, Inc., CA, respectively), Nitrite (NO_2^-) and nitrate (NO_3^-) levels were measured using the Nitrite/Nitrate assay kit (Cayman Chemical Co., Ann Arbor, MI) on the basis of the Griess reaction.

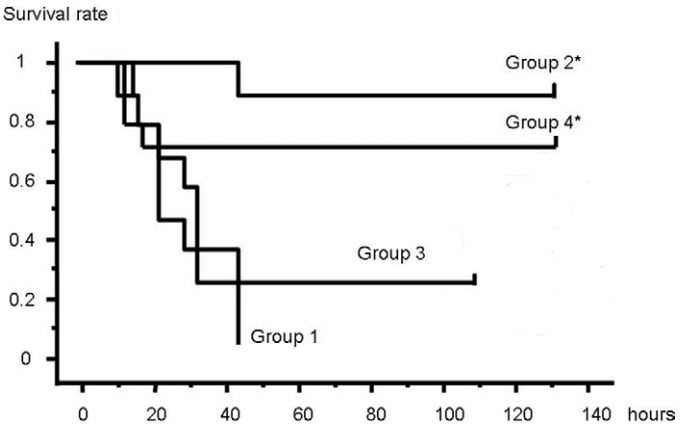

FIG. 1. Survival rate of each group after treatment with a lethal dose of LPS. $P < 0.05$ *versus* Group 1 rats.

Data Analysis

Survival curves were calculated using the Kaplan–Meier method, and survival was compared using generalized Wilcoxon test. Statistical analysis was performed using Fisher exact test and the Mann–Whitney *t*-test. Significance was defined as $P < 0.05$. All values were expressed as mean ± SD.

RESULTS

Survival Rate

Fig. 1 shows the survival rate of each group after treatment with a lethal dose of LPS. In Group 1, all animals died within 48 h after LPS injection, whereas 9 of 10 rats in Group 2 survived the lethal dose of LPS. The survival rate was significantly higher than that in Group 1. Eight of 10 rats in Group 3 died within 48 h; however, 7 of 10 rats in Group 4 survived the lethal dose of LPS. The survival rates in Groups 2 and 4 were significantly higher than those in Groups 1 and 3.

Endotoxin Level in the Portal Vein

The endogenous endotoxin level in the portal vein in Group 2 rose over 50 pg/mL after PTO (54.2 ± 46.0 pg/mL), while those in Groups 1, 3, and 4 were undetectable (Fig. 2).

The Level of NO_2^-/NO_3^-, IL-6, and TNF-α

After administration of a lethal dose of LPS, the levels of NO_2^-/NO_3^- increased continuously in every group (Fig. 3). The level of plasma NO_2^-/NO_3^- in Group 1 6 h after the administration of LPS was significantly higher than those in Groups 2, 3, and 4. At 9 h, the levels of plasma NO_2^-/NO_3^- in Group 1 were significantly higher than those in Groups 2 and 4. Regarding IL-6, the level of IL-6 in Group 1 was significantly higher than those in Groups 2, 3, and 4 at 6 and 9 h after LPS administration (Fig. 4). Regarding TNF-α, the levels in Groups 1 and 3 were similarly elevated to