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Nitric Oxide and Tumor Necrosis Factor-α Production of Kupffer Cells In Rats with Obstructive Jaundice and Its Relief by External Biliary Drainage

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Abstract

Background: Preoperative biliary drainage in obstructive jaundice has been the subject of controversy and the role of Kupffer cells in obstructive jaundice is still unclear. The aim of this study was to investigate the production and time course of nitric oxide (NO) and tumor necrosis factor-α (TNFα) released from Kupffer cells in obstructive jaundice.

Methods: Nitrite (NO_2^-) as the stable end product of NO production of Kupffer cells in response to lipopolysaccharide (LPS) was measured together with TNFα production in bile duct ligated (BDL) rats and in its relieved rats by external biliary drainage.

Results: In systemic endotoxemia of BDL rats, plasma levels of nitrite/nitrate and TNFα were significantly elevated, and returned to the levels of sham-operated control rats in 7 to 14 days after external biliary drainage. The time course of TNFα production showed its peak level 3 hours after incubation of isolated Kupffer cells with 1 μg/ml LPS. Kupffer cells isolated from BDL rats produced 2-fold greater amounts of nitrite and TNFα than those from sham-operated control rats in response to LPS stimulation, and its nitrite accumulation increased in a time dependent manner. After external biliary drainage, nitrite production by Kupffer cells isolated from BDL rats and its dependence on LPS decreased as well as TNFα production.

Conclusions: These results indicate that Kupffer cells play a key role through the production of NO and TNFα in the development of pathogenesis of obstructive jaundice, and preoperative biliary drainage in obstructive jaundice might be significant due to decrease of detrimental amount of NO and TNFα.

Key words: Rat, bile duct ligation, obstructive jaundice, Kupffer cell, lipopolysaccharide, nitric oxide, tumor necrosis factor-α external biliary drainage

Listing of Abbreviations

NO - nitric oxide, NOS - nitric oxide synthese, iNOS - inducible NOS, TNFα - tumor necrosis factor-α, LPS - lipopolysaccharide, BDL - bile duct ligated, ED - external biliary drainage, Sham - sham-operated control, PBD - preoperative biliary drainage

Introduction

Obstructive jaundice in patients who undergo surgery leads to a high rate of postoperative morbidity and mortality[1)] due to renal failure[2)], gastrointestinal bleeding, coagulopathy[3)], cardiovascular instability manifested as systemic hypotension[4)], and immune suppression. These complications are closely related to gram-negative sepsis and systemic endotoxemia is integral to the pathophysiology of obstructive jaundice[5)]. In fact, endotoxins were positive in 11 of 13 jaundiced patients before, during, and after surgery[6)].

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However, the role of preoperative biliary drainage (PBD) in obstructive jaundice has been the subject of controversy[7-9] although it is supposed that PBD such as percutaneous transhepatic drainage or endoscopic naso-biliary drainage is clinically common procedure for obstructive jaundiced patients for radical operation. Because there is no evidence in the literature to support the view that routine PBD improves postoperative morbidity and mortality in patients with obstructive jaundice undergoing resection[8].

Kupffer cells are mononuclear cells resident in the hepatic sinusoids. They account for about one third of the non-parenchymal cells within the liver and constitute 90% of the cells of the mononuclear phagocytic system. Kupffer cells isolated from animals with obstructive jaundice are known to secrete chemical mediators such as cytokines (IL-1, IL-6, TNFα), prostaglandins (PGE2), and oxygen radicals in response to various stimuli [10,11]. Kupffer cells may have a key role in the development of endotoxemia in obstructive jaundice[5].

Nitric oxide (NO), a short-lived unique biologically active molecule, has been implicated in numerous physiologic and pathophysiologic processes. Particularly, the role of NO in sepsis has been discussed by many authors, but question remains concerning whether the effect of endogenous NO on the stressed organism is beneficial or detrimental[12]. In rat hepatic ischemia-reperfusion injury, Kupffer cells might be the major source of the induction of inducible NO[13]. In addition, inflammation in the liver is a complex interaction between parenchymal and non-parenchymal cells caused by cytokines produced by Kupffer cells[14]. Kupffer cells in rats with obstructive jaundice produce a greater amount of superoxide and NO anions, which may be responsible for the impaired liver function[15].

In the present study, we investigated the specific function of the Kupffer cells to produce NO and TNFα in response to lipopolysaccharide (LPS) stimulation using the method of primary culture of Kupffer cells isolated from BDL rats and relieved rats by ED.

Materials and Methods

Animals:

All experiments were performed on male Wistar rats weighing 250 to 300 g. The animals were subjected to a 12-hour artificial daylight cycle and allowed free access to food and water. After the placement of an external drainage catheter, they were housed in individual cages to protect the catheter. All surgical procedures were carried out under ether and pentobarbital anesthesia and clean, but not sterile conditions. The use of animals in this research complied with all relevant guidelines of the Japanese government and Kagoshima University.

Bile duct ligation and External biliary drainage:

Through a 2 cm upper median incision the common bile duct was mobilized, a polyethylene tube with an outer diameter of 0.61 mm (PE10, Becton Dickinson , New Jersey, USA) was inserted into the common bile duct according to the cut-down technique[16], secured using silk ties placed around the proximal bile duct, and distal bile duct was divided. The free end of the tube was passed through a subcutaneous tunnel to the nape and fixed. Obstructive jaundice and external biliary drainage were performed by ligating or releasing the end of the tube. The rats with external biliary drainage received a daily subcutaneous injection of 10 ml of 0.9% sodium chloride to replace fluid loss due to biliary drainage.

Experimental groups:

The rats were divided into five groups: (1) bile duct ligated (BDL) group (n=10); (2) external biliary drainage (ED) groups for 3 days (ED 3, n=6); (3) 7 days (ED 7, n=6); (4) 14 days (ED 14, n=6); and (5) sham-operated (Sham) control group (n=10). In the BDL group, rats were jaundiced by ligating the end of the tube for 14 days. Rats in the ED 3, ED 7, and ED 14 groups had bile duct ligation for 14 days followed by external biliary drainage, by releasing the end of the tube, for additional 3, 7, and 14 day periods, respectively. In sham-operated rats (Sham) the common bile duct was mobilized but not ligated.

Plasma assays:

Blood samples were collected by cardiac puncture, and plasma was collected and stored at -80℃ until analysis. The liver function tests (total bilirubin, alanine aminotransferase, and alkaline phosphatase) were measured by standard methods to determine the degree of hepatic injury caused by obstructive jaundice and the recovery of hepatic function by external biliary drainage.

Isolation and culture of Kupffer cells:

Kupffer cells were isolated from rat livers using a modified *in situ* collagenase perfusion method as described by Munthe-Kaas[17], and an adhesion technique as described by Fujita et al.[18] After laparotomy, the liver was perfused thorough the portal vein with Ca^{2+}-free Hank's balanced salt solution (HBSS(-)) containing 5 mM EGTA (Kyokuto Pharm., Tokyo, Japan) at 37℃ for 5 min at a flow rate of 30 ml/min, followed by a 5 min perfusion with HBSS(+) supplemented with 0.05% collagenase (Wako Pure Chemical Industries, Tokyo, Japan) at the same flow rate. Then the liver was removed *en bloc* and minced. The liver fragments were incubated in 80 ml HBSS(+) containing 0.01% collagenase and 100 mM HEPES for 45 min at 37℃(pH 7.3-7.4) with continuous stirring. During the incubation, 20 mg/ml deoxyribonuclease 1 (Sigma, St. Louis, MO, USA) was added to aid in digestion of extracellular debris[19]. The suspension was filtered through a stainless steel mesh and the filtrate was centrifuged at 600 rpm for 1 min. The resulting pellet consisted of parenchymal cells. Non-parenchymal cells (NPC) in the supernatant were collected by centrifugation at 1500 rpm for 7 min. The NPC pellet was washed three times with HBSS(+). The final NPC suspension was freed of erythrocytes and cell debris by density centrifugation with Histopaque-1083 (Sigma, St. Louis, MO, USA) at 2,600 rpm for 5 min at 4℃. Collected interface cells were washed three times with HBSS(+). The purified NPC pellet was resuspended in warm Eagle's minimal essential medium (MEM) without phenol red (Nissui, Tokyo, Japan) supplemented with 10% FCS (Mitsubishi Kasei Corp., Tokyo, Japan) and 1% Penicillin/Streptomycin (PC/SM) and then plated in uncoated 10 cm plastic dish at a concentration of 2 x 10^6 cells/ml. After 15 min incubation in a humidified incubator at 37℃ under 5% CO_2 in air, the dish was washed with HBSS(+) to detach the non-adherent cells, leaving the adherent cells, which were Kupffer cells[17]. The Kupffer cells remaining in the dish were recovered by incubation with PBS containing 0.02% EGTA and 2% FCS for 40 min at 4℃ and collected with cell-scraper[18]. Recovered Kupffer cells were washed three times and resuspended in Eagle's MEM with 10% FCS. Then 5 x 10^5 of Kupffer cells in 1 ml medium were replated in 24-well culture plate. 10-15 x 10^6 of Kupffer cells were obtained from each liver. The viability of the cells, as measured by the Trypan blue exclusion test, was more than 95%. And the purity of the cells was more than 90% identified by light microscopy and by their phagocytosis of latex beads (3 μm). After 24 hours of in vitro culture in Eagle's MEM without phenol red containing 10% FCS and 1% PC/SM, the culture medium was changed and various concentration of LPS (from E. Coli, O111:B4; Sigma, St. Louis, MO, USA) were added. After stimulation with or without LPS, the cells were incubated for 48 hours in a humidified incubator at 37℃ under 5% CO_2 in air. Aliquots of the medium were collected at 1, 3, 6, 12, 24, and 48 hours after stimulation, and stored at -80℃ after centrifugation until analysis.

Determination of Nitric Oxide:

Nitric oxide formation from cultured Kupffer cells was measured as NO_2^- (Nitrite),the stable end product of NO, accumulated in the cell media, which reflects the activity of NO syntheses of Kupffer cells[20]. Nitrite was determined spectrophotometrically by using the Griess reaction[21]. Briefly, 100 ml of Griess reagent (1 part 0.1% naphthylethylenediamine dihydrochloride in distilled water plus 1 part 1% sulfanilamide in 5% concentrated H_3PO_4), and 100 μl of supernatant was applied to each well of a microtiter plate; the pink azo dye was quantitated by a microplate reader (Labsystems iEMS Reader MF) at 550 nm. Plasma nitrite/nitrate concentration was determined by the Griess reaction after reduction of nitrate to nitrite by modified method of Schmidt et al.[22]. Before analysis, plasma was deproteinized by the addition of 300 μl 0.3 N NaOH and 400 μl H_2O to a 100 μl plasma sample. Treated samples were mixed and allowed to react for 5 min at room temperature, and 300 μl 5% (W/V) $ZnSO_4 \cdot 7H_2O$ was added and allowed to react for an additional 5 min at room temperature. The treated samples were centrifuged at 2,800 g for 10 min. Nitrate was stoichometrically reduced to nitrite by incubation of 100 μl of supernatant for 15 min at 37℃, in the presence of 0.5 u/ml nitrate reductase [NAD(P)H: nitrate oxidoreductase from Aspergillus species; Boehlinger-Mannheim], 250 mM NADPH, and 25 mM FAD, in a final volume of 160 μl. When nitrate reduction was complete, NADPH, which interfered with the following nitrite determination, was oxidized with 50 u/ml lactate dehydrogenase (rabbit muscle; Boehlinger-Mannheim) and 50 mM sodium pyruvate, in a final volume of 170 μl, with incubation for 5 min at 37℃. Standards were prepared using $NaNO_2$ and $NaNO_3$

ranging from 5 to 50 mM. Samples were assayed in triplicate. Data presented in the figures and tables were expressed as nitrite/nitrate in nanomoles per milliliter for plasma samples and nitrite in nanomoles per well (5×10^5 Kupffer cells) for supernatant samples, respectively.

Determination of TNFα by Enzyme-Linked Immunosorbent Assay:

TNFα concentration of plasma and supernatant was measured with a rat TNFα ELISA kit (Factor-Test-X, Genzyme, Cambridge, MA) as described by Kurose et al.[23]. Briefly, the standards and samples were added to microtiter wells, precoated with anti-rat TNFα monoclonal antibody, and incubated for 2 hours at 37℃. After washing, horseradish peroxidase-conjugated polyclonal anti-TNFα was added into each well, and then the plate was covered and incubated for 1 hour at 37℃. After washing the plate 4 times, tetramethylbenzimine-containing buffer was added to develop the horseradish peroxidase-associated yellow color. The absorbance at 450 nm was recorded, and the TNFα level was determined from standard plots, which were made by using various concentration (35-2,240pg/ml) of recombinant rat TNFα. The ELISA had a lower detection limit of 10 pg/ml. The ELISA recognizes free TNFα as well as TNF bound to binding proteins (immunologically detectable TNFα).

Determination of plasma endotoxin:

Plasma endotoxin concentration was quantitatively measured with Endotoxin-specific test (Endospecy kit; Seikagaku Kogyo Co., Tokyo, Japan) by an endotoxin-specific assay using recombined limulus coagulation enzymes as described by Obayashi et al.[24]. Sterile endotoxin-free disposable ware was used throughout to prevent contamination of blood samples. Interfering factors in plasma were removed by exposing 100 µl of plasma to 200 µl of 0.32 M perchloric acid at 37℃ for 20 min, followed by removal of the denatured material by centrifugation at 3,000 rpm for 10 min. Perchloric acid pretreatment can completely eliminate false positive substances, including bile salts, in blood samples. A 25 µl aliquot of the supernatant was neutralized with an equal volume of 0.18 M NaOH, then subjected to the assay. The sample was added to a 50 µl aliquot of endotoxin-specific test dissolved in 0.2 M Tris-HCl buffer, pH 8.0, and the mixture was incubated at 37℃ for 30 min. Absorbance was measured at 545 nm after diazotization to avoid interference from bilirubin and other yellow pigments. Sensitivity was enhanced by adding 50 µl of each of the following reagents: 0.04% (w/v) sodium nitrite in 1M HCl, 0.3% (w/v) ammonium sulfate in distilled water, and 0.07% (w/v) N-1-naphthylethylenediamine dihydrochloride in 14% N-methyl-2-pyrrolidone solution. Linearity was obtained between the endotoxin concentration and absorbance with a sensitivity of 1 pg/ml of *Escherichia coli* O111:B4 endotoxin.

Statistical analysis:

All results were expressed as mean±SD, and the data were analyzed using standard statistical analysis, i.e., one-way ANOVA and Fisher's post-hoc test for comparing multiple groups. A *p*-value of less than 0.05 was considered to be significant.

Results

Liver functions

As shown in Table 1, plasma total bilirubin, ALT (alanine aminotransferase), and ALP (alkaline phosphata-

Table 1. Changes in function tests following bile duct ligation (BDL) and external biliary drainage (ED)

Group	*n*	*Total Bilirubin (mg/dl)*	*ALT (IU/l)*	*ALP (IU/l)*
BDL	10	6.7±2.6*	179±63*	1,152±594*
ED 3	6	1.9±0.8*†	111±50*	870±120*
ED 7	6	0.4±0.1*†	83±15*†	762±151
ED14	6	0.3±0.1 †	49± 6 †	546± 97
Sham	10	0.2±0	40± 8	532±176

Values are mean ± SD.BDL, bile duct ligation group;
ED, external biliary drainage group; Sham,sham - operated control group;
ALT, alanine aminotransferase; ALP, alkaline phosphatase.
*p<0.01vs. Sham group. †p<0.01vs. BDL group.

Table 2. Changes in plasma endotoxin, TNF α , and NO_2^-/NO_3^- following bile duct ligation (BDL) and external biliary drainage (ED)

Group	*n*	*Endotoxin (pg/ml)*	*TNF - α (pg/ml)*	*NO_2-/NO_3- (nmol/ml)*
BDL	10	102.1±30.8*	53.8±25.5*	45.1±12.8*
ED 3	6	20.0±10.1*†	20.9±12.1*	37.9± 8.2*
ED 7	6	6.6± 5.5 †	13.2± 7.5 †	26.3± 4.5 †
ED14	6	6.3± 2.1 †	9.6± 3.2 †	22.6± 4.1 †
Sham	10	5.8± 1.9	7.7± 1.3	20.9± 5.0

Values are mean ± SD.BDL, bile duct ligation group; ED,external biliary drainage group; Sham, sham - operated control group; TNF - α , tumor necrosis factor - α ; NO_2^-/NO_3^-, nitrite/nitrate as the end product of nitric oxide in blood.
*p<0.01vs. Sham group. †p<0.01vs. BDL group.

se) levels were significantly elevated in the BDL group, and rapidly decreased after external biliary drainage. The mortality of each group was 0%.

Plasma concentrations of endotoxin, TNFα, and nitrite/nitrate

Plasma endotoxin, TNFα and nitrite/nitrate levels were significantly elevated in the BDL group, and then rapidly decreased at ED 3 and returned almost to control levels at ED 7 or ED 14 (Table 2).

TNFα production by Kupffer cells

When Kupffer cells isolated from both sham and BDL groups were incubated with 1 μg/ml LPS, TNFα was released from Kupffer cells of each model in a time dependent manner. The time course of TNFα production from Kupffer cells showed the peak level at 3 hours followed by a gradual decrease in both sham and BDL groups, although Kupffer cells without LPS stimulation released little amount of TNFα in both sham and BDL

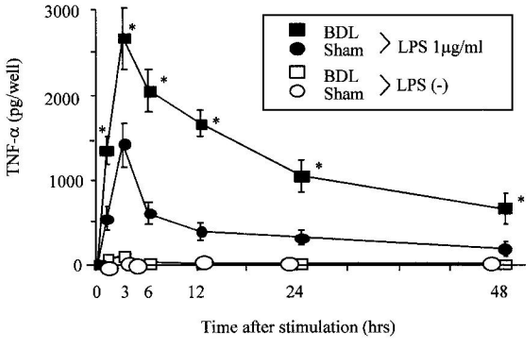

Fig. 1. The time course of TNFα production by Kupffer cells isolated from BDL and Sham rats with 1 μg/ml LPS, and from BDL and Sham rats without LPS stimulation for 48 hours (*p< 0.01 vs. Sham group). Each point represents the mean ± SD.
BDL, bile duct ligation group; Sham, sham-operated control group; LPS, lipopolysaccharide; TNFα , tumor necrosis factor-α

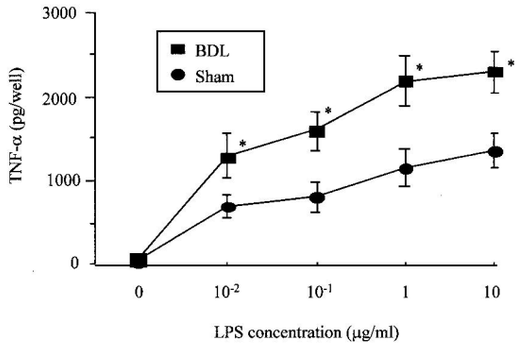

Fig. 2. Dose-response curve of TNFα production by Kupffer cells isolated from BDL and Sham rats 3 hours after incubation with or without various concentration of LPS (*p< 0.01 vs. Sham group). Each point represents the mean ± SD.
BDL, bile duct ligation group; Sham, sham-operated control group; LPS, lipopolysaccharide; TNFα , tumor necrosis factor-α

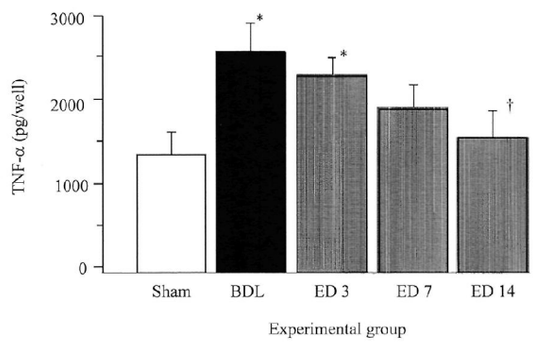

Fig. 3. Comparison of TNFα levels of each group 3 hours after incubation of Kupffer cells with 1 μg/ml LPS (*p< 0.01 vs. Sham group, †p< 0.01 vs. BDL group). Each column represents the mean ± SD. Sham, sham-operated control group; BDL, bile duct ligation group; ED, external biliary drainage group; LPS, lipopolysaccharide; TNFα , tumor necrosis factor-α ; NO_2^-, nitrite as the end product of nitric oxide in culture media.

groups. The response to LPS stimulation in the BDL group was greater than that in sham group, and its peak level of BDL group was 2-fold higher than that of sham group (Fig. 1). The hypersensitivity of TNFα production by Kupffer cells from BDL rats responded to LPS stimulation (Fig. 2). Significantly elevated TNFα level in the BDL group decreased gradually with external biliary drainage, and statistically returned to control levels in ED 14 (Fig. 3).

Nitric oxide production by Kupffer cells

When Kupffer cells isolated from both BDL and Sham groups were stimulated with 1 μg/ml LPS, nitrite was released from Kupffer cells of each model in a time dependent manner. The response to LPS stimulation in the BDL group was 2-fold greater than that in the sham group (Fig. 4), and the response curve returned to the level of the sham group level when the period of external biliary drainage was extended at ED 7 or 14. The nitrite production in response to increased concentration of LPS showed a dose-dependent manner (Fig. 5). In a comparison of nitrite levels released from Kupffer cells in each group 48 hours after stimulation with 1 μg/ml LPS, significantly elevated nitrite level in the BDL group decreased gradually with external biliary drainage, and statistically returned to control levels at ED 7 and 14 (Fig. 6). NO production by Kupffer cells from BDL rats responded to LPS stimulation and its dependence on LPS decreased gradually with biliary drainage as well as TNFα production.

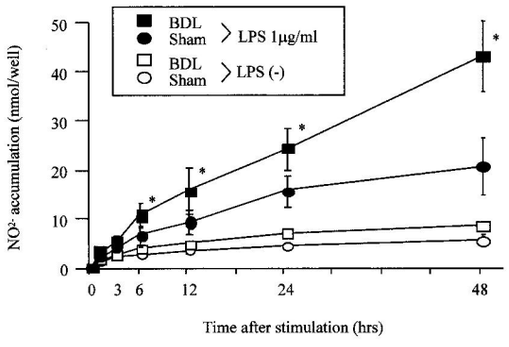

Fig. 4. Time course of NO_2^- production from Kupffer cells isolated from BDL and Sham rats with 1 μg/ml LPS, and from BDL and Sham rats without LPS stimulation for 48 hours (*p<0.01 vs. Sham group). Each point represents the mean ± SD. BDL, bile duct ligation group; Sham, sham-operated control group; LPS, lipopolysaccharide; NO_2^-, nitrite as the end product of nitric oxide in culture media.

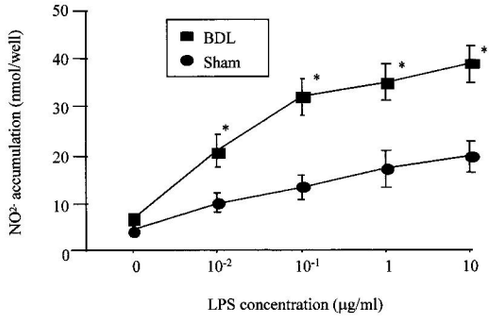

Fig. 5. Dose-response curve of NO_2^- production by Kupffer cells isolated from BDL and Sham rats 48 hours after incubation with or without various concentration of LPS (*p<0.01 vs. Sham group). Each point represents the mean ± SD. BDL, bile duct ligation group; Sham, sham-operated control group; LPS, lipopolysaccharide; NO_2^-, nitrite as the end product of nitric oxide in culture media.

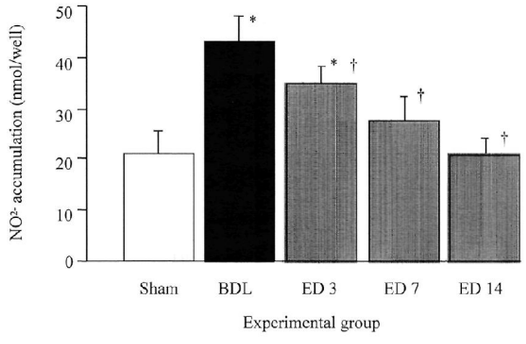

Fig. 6. Comparison of NO_2^- levels of each group 48 hours after incubation of Kupffer cells with 1 μg/ml LPS (*p<0.01 vs. Sham group. † p<0.01 vs. BDL group). Each column represents the mean ± SD. Sham, sham-operated control group; BDL, bile duct ligation group; ED, external biliary drainage group; LPS, lipopolysaccharide; NO_2^-, nitrite as the end product of nitric oxide in culture media.

Discussion

In Japan, almost all surgeons agree that biliary decompression should be performed prior to major surgery such as pancreatoduodenectomy or hepatectomy[25]. However, the role of PBD in obstructive jaundice has been the subject of controversy[7-9]. Because there is no evidence in the literature to support the view that routine PBD improves postoperative morbidity and mortality in patients with obstructive jaundice undergoing resection[8].

The present study has demonstrated that production of NO and TNFα by Kupffer cells was significantly

increased in rats with obstructive jaundice. Kupffer cells isolated from rats with bile duct ligation manifested the releasing NO and TNF compared to sham- operated rats when challenging with LPS. Then, the systemic endotoxemia was seen in bile duct ligated rats and recovery was seen with external biliary drainage. The systemic endotoxemia developing after the relief of biliary obstruction by external drainage might contribute to increased mortality[26]. By contrast, the relief of obstruction by both external and internal drainages prevented the endotoxin-related death in experimentally jaundiced rats[27]. Additionally, biliary obstruction was a more important factor than gastrointestinal bile flow in the development and reversal of endotoxemia[27].

Kupffer cells have a central role in the development of endotoxemia in obstructive jaundice[5], because there are two hypotheses for the mechanism of systemic endotoxemia. First, the absence of intraluminal bile salts promotes bacterial overgrowth and translocation of bacteria and endotoxin from the large intestine into the portal circulation[28]. Second, the depressed Kupffer cell clearance capacity in the liver permits spillover of endotoxin into the systemic circulation[29]. Therefore, investigation of the specific function of the Kupffer cell population is essential to elucidate the mechanism of the pathophysiology of obstructive jaundice.

Biliary obstruction in mice elevated circulating TNFα and interleukin-6 (IL-6) levels and peritoneal macrophages isolated from BDL mice produced significantly higher amounts of IL-6 and TNFα in response to LPS stimulation[11]. Human peripheral monocyte isolated from jaundiced patients produced significantly higher amounts of TNFα and IL-6[30]. TNFα is known to be a major carrier of the LPS-evoked biological effects in animals. Infusions of recombinant TNFα have been shown to induce cardiovascular shock, hemorrhagic necrosis in tissues, and metabolic derangements similar to both experimental endotoxemia and clinical septic shock. The importance of TNFα in sepsis is supported further by studies showing that passive immunization with antibodies against TNFα protects against the lethal effects of LPS and bacterial administration[31, 32]. In the present study, Kupffer cells isolated from BDL rats released much higher amounts of TNFα in response to LPS stimulation than those from sham-operated rats as well as higher concentrations of plasma TNFα from BDL rats compared to sham-operated rats. These results suggest that immune dysfunction caused by too much production of TNFα by an activated state of macrophage and the circulating immune regulatory mediators are closely related to the postoperative complications observed in jaundiced patients.

Nitric oxide (NO) is a short-lived free radical that plays an important regulatory role in several biological processes[33]. The formation of NO from L-arginine and molecular oxygen is catalyzed by the enzyme nitric oxide syntheses (NOS). The abilities of NO to dilate blood vessels, block platelet and leukocyte adhesion to endothelial cells, act against microbes, and scavenge superoxide suggest that increased production of NO acts to maintain microvascular blood flow and protect the endothelium from oxidative stress and damage[34-36]. NO production stimulates various intracellular reactions, resulting in several physiological responses. In contrast, LPS or various cytokines expresses inducible NOS (iNOS) as the third NOS isoform after induction[37]. In the present study, the phenomenon observed that NO production from Kupffer cells in response to LPS stimulation was enhanced in obstructive jaundice and then recovered to control levels with external biliary drainage may represent a natural reaction to compensate disorders in obstructive jaundice.

In conclusion, excess production of NO as well as TNFα by Kupffer cell activation in obstructive jaundice might be detrimental, suggesting that both NO and TNFα released from Kupffer cells play a key role in the pathogenesis of obstructive jaundice, and PBD is significant due to the suppression of Kupffer cell activation.

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ラット閉塞性黄疸におけるKupffer細胞の一酸化窒素（NO）およびTumor Necrosis Factor－α（TNFα）産生能と減黄による影響の検討

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【目的】閉塞性黄疸（閉黄）患者の外科手術に際しては諸合併症により術後死亡率が高いため，臨床においては術前減黄処置が一般的であるが，その功罪はいまだ結論が出ていない。閉黄における諸病態の本態はエンドトキシン血症に起因するもので，肝 Kupffer 細胞（KC）がその発生に重要な役割を演じているといわれている。われわれは閉黄および閉黄後減黄したラット肝からそれぞれ KC を分離培養し，エンドトキシン（LPS）刺激に対する一酸化窒素（NO）および Tumor Necrosis Factor（TNFα）の産生能を検討した。

【対象・方法】ラット総胆管内にポリエチレンチューブを留置後末梢を切離してチューブ断端を項部に固定，その断端を結紮して14日間経過した閉黄群と，その後断端を開放して３，７，14日間経過した減黄群を作成した。各群の血液を採取して血漿中エンドトキシン，TNFα，NO_2^-／NO_3^-を測定。同時に肝組織より cpllagenage 灌流法と遠心分離法にて分離採取した KC を48時間初代培養して，lipopolysaccharide（LPS）を添加刺激後経時的に採取された上清中の TNFα，NO_2^-を測定した。

【結果】①血漿中エンドトキシン，血漿中 TNFα，NO_2^-／NO_3^-は閉黄群で有意に上昇し減黄７日以降で sham 群レベルまで回復した。②分離培養された KC の LPS に対する TNFα 産生は刺激３時間後にピークがみられた。③LPS 刺激に対して閉黄群の肝 KC は sham 群の２倍の TNFα，NO_2^-を産生したが，減黄７日以降で sham 群レベルまで回復した。

【結論】閉黄によるエンドトキシン血症により肝 KC が活性化され，本来侵襲に対する生体防御に働くべき NO や TNFα の過剰産生を引き起こすことが閉黄時の病態発現に大きな役割をになうこと，またその回避のために術前減黄は重要であることが推測された。