MICROBIAL PATHOGENICITY

Promotion of bacterial translocation by major liver resection in obstructive jaundice in rats colonised predominantly with indigenous *Escherichia coli*

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The influence of major liver resection in obstructive jaundice on bacterial translocation was evaluated in rats that were colonised predominantly with a genetically labelled strain of *Escherichia coli*. The strain, JNW14, originally isolated from rat faeces, was labelled with bacitracin, neomycin and streptomycin resistance markers. Fifty-two specific-pathogen-free male Wistar rats were divided into three experimental groups and were treated as follows: group 1 (n = 8), sham ligation of common bile duct; group 2 (n = 7), common bile duct ligation (CBDL); and group 3 (n = 37), 70% hepatectomy 7 days after CBDL. The rats were treated with the above antibiotics and then given *E. coli* strain JNW14 in their drinking water. Translocation of *E. coli* JNW14 from the gastrointestinal tract to the mesenteric lymph nodes (MLNs), lungs, liver, spleen and portal vein was evaluated in each group. In group 3 (CBDL plus hepatectomy), the incidence of translocation of *E. coli* JNW14 to the liver and spleen after hepatectomy was significantly higher than in groups 1 and 2. This result indicates that major liver resection in obstructive jaundice promotes bacterial translocation to systemic organs. Furthermore, the numbers of viable *E. coli* JNW14 in the MLNs in the lung culture-positive rats were significantly higher than those in the lung culture-negative rats, suggesting that lymphatic-thoracic duct systemic circulation is a major route of bacterial translocation.

Introduction

Advances in surgical techniques have enabled more extensive hepatobiliary operations in jaundiced patients and these advances have contributed to the reduction in mortality in the early stages of hepatobiliary diseases. However, bacterial infections are still frequently observed as complications in patients who have undergone liver resection and in patients with obstructive jaundice [1, 2]. Control of infection and endotoxaemia is an important factor determining morbidity and mortality in these patients [3–8]. The mechanism by which enteric bacteria reach an infective focus has not been entirely elucidated. Gram-negative enteric bacteria are isolated commonly from patients with infective complications after hepatobiliary operations [9, 10], but in >30% of patients with sepsicaemia and multiple organ failure, the origin of the bacteria could not be identified even though intensive clinical examinations or autopsies had been performed [11, 12]. Recent studies have shown that, under certain conditions, enteric bacteria in the gastrointestinal tract enter the epithelial mucosa and invade the lamina propria and then spread to the mesenteric lymph nodes (MLNs) and other organs. This condition has been described as ‘bacterial translocation’ [13]. These results indicate that indigenous intestinal microflora are a possible origin of systemic infections that occur after surgical operations. Gut barrier failure and subsequent translocation of enteric bacteria to other organs are considered to be a major cause of systemic infection resulting in multiple organ failure [14]. Bacterial translocation has been demonstrated in several experiments and clinical studies in cases of burns [15, 16], haemorrhagic shock [17], endotoxaemia [18, 19], intestinal obstruction [20], immunosuppression [21], inflammatory bowel disease [22] and total parenteral nutrition [23]. Furthermore, it has been shown that major liver resection and...
obstructive jaundice induce translocation of enteric bacteria [24–28].

Steffen and Berg [29] found a positive relationship between overgrowth of intestinal bacteria and bacterial translocation in experiments with bacteria possessing an antibiotic resistance marker. An increase in the number of certain intestinal bacteria induced by oral administration of antibiotics allowed them to translocate into the MLNs [30]. Reynolds et al. [31] have shown that the degree of bacterial translocation was increased by operative trauma (bowel handling) in an experimental model of obstructive jaundice. However, no difference was observed between the incidences of translocation in bile duct-ligated rats that had undergone further surgical trauma and those that had not. It remains unclear whether more severe trauma, such as liver resection, which is a common procedure in jaundiced patients, promotes bacterial translocation in patients with obstructive jaundice. The aim of this study was to determine the influence of major liver resection in obstructive jaundice on bacterial translocation in rats.

Materials and methods
Isolation of an E. coli mutant strain carrying drug resistance markers
E. coli strains were isolated from faeces of a specific-pathogen-free Wistar rat and their identity was confirmed by the API20E system (bioMérieux, France). One of the E. coli strains was cultured overnight in 10 ml of Gifu Anaerobic Medium (GAM, Nissui Pharmaceutical, Tokyo, Japan). Cells were collected by centrifugation, resuspended in 1.0 ml of freshly prepared GAM broth, and 0.1 ml of this suspension was then placed on a GAM agar plate containing streptomycin 25 mg/L and neomycin 25 mg/L of each. The plates were incubated at 37°C for 48 h. One of the streptomycin-resistant colonies was isolated. Colonies of this strain resistant to neomycin and bacitracin were isolated in the same manner. Thus, a bacitracin-neomycin-streptomycin-resistant strain of E. coli was derived and named strain JNW14.

Experimental animals and design
Fifty-two 8-week-old male specific-pathogen-free Wistar rats weighing 200–250 g (Japan SLC, Hamamatsu, Japan) were used in this study. Before each experiment the animals were fed standard rat chow and provided with tap water ad libitum. The rats were acclimatised to laboratory conditions for 4–5 days. They were housed in plastic cages under constant conditions of temperature (25 ± 2°C) and humidity (60–70%) with a 12.5-h light/11.5-h dark cycle. They were divided into three experimental groups. Group 1 rats (n = 8) were subjected to sham operations in which their common bile ducts were separated from the surrounding tissues without ligation and division. Group 2 (n = 7) and 3 (n = 37) rats were subjected to ligation of their common bile ducts (CBDL) at three sites with 5-O silk. The common bile ducts were then cut at the point between the middle and distal ligations. Seven days after ligation of their common bile ducts, group 3 rats (n = 37) were subjected to a 70% hepatectomy as described previously by Wang et al. [26]. All three groups of rats were given drinking water containing 2 g/L each of streptomycin sulphate, neomycin sulphate and bacitracin sulphate for 4 days after the sham or CBDL operation, then given ad libitum overnight cultures of E. coli JNW14 grown in GAM broth as drinking water for 3 days; then rats in group 3 underwent hepatectomy. All operations were performed aseptically under light ether anaesthesia. When rats were killed, 1.0 ml of blood sample was collected from each animal by cardiac puncture, and serum levels of bilirubin, alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were determined to evaluate the liver function of rats in each group.

All animal procedures complied with animal care guidelines of the Institute of Animal Experimentation, School of Medicine, The University of Tokushima.

Translocation of E. coli JNW14
Bacterial translocation in groups 1 and 2 rats was evaluated 7 days after the operation. Rats in group 3 (seven or eight rats per sampling time) were examined at 3, 6, 12, 24 and 48 h after the hepatectomy. An incision was made with sterile instruments through the skin and peritoneum of the abdomen. The MLNs, liver, lungs and spleen were sampled aseptically and then transferred to pre-weighed tubes containing an anaerobic solution (KH2PO4 0.45%, Na2HPO4 0.6%, L-cysteine hydrochloride 0.05%, Tween 80 0.05% and agar 0.01% in water). Portal vein blood was also collected. The organs were weighed and then homogenised with Teflon grinders. A 0.1-ml sample of homogenate or of portal vein blood was spread on two GAM agar plates containing bacitracin 30 mg/L and streptomycin and neomycin, 25 mg/L each. The plates were incubated at 37°C for 48 h to detect E. coli JNW14. Further 0.1-ml samples of organ homogenates and blood samples were plated on two GAM agar plates, for aerobic and anaerobic incubation, and the plates were incubated at 37°C for 48 h. Anaerobic cultivation was achieved using a GasPak system (Becton Dickson and Company, Cockeysville, MD, USA). The numbers of viable bacteria were calculated as cfu/g of organ.

Caecal contents were also collected, transferred to pre-weighed tubes, and suspended in an anaerobic solution. Serial dilutions with anaerobic solution were placed on GAM agar plates (anaerobic incubation for total bacterial counts) and GAM plates containing bacitracin, streptomycin and neomycin as above (aerobic incubation for counts of E. coli JNW).
Colonies growing on antibiotic-containing plates were identified as *E. coli* on the basis of colony morphology, Gram’s staining and biochemical properties in the API20E system.

**Statistical analysis**

Translocation incidence was evaluated by Fisher’s exact test. Other data were analysed by Student’s *t* test.

**Results**

All rats subjected to CBDL (groups 2 and 3) were deeply jaundiced and the proximal remnants of the common bile duct were markedly dilated above the ligature. Serum levels of total bilirubin, direct bilirubin, alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were significantly elevated in group 2 (CBDL) and group 3 (CBDL plus hepatectomy) compared with those in group 1 (sham operation). ALAT and ASAT levels at 12 h after hepatectomy and the total bilirubin level at 24 h after hepatectomy were significantly increased compared with those in CBDL rats (Table 1).

Total bacterial counts in the caecum of the rats in each group are shown in Table 2. In group 3 (CBDL plus hepatectomy), the total bacterial number in the caecum was determined at 12 h after hepatectomy. Although the total bacterial number was not significantly different among the groups, the number of caecal bacteria of the rats in the CBDL group tended to be higher than in the other groups due to the overgrowth of *E. coli* JNW14 in the caecum. In the rats of all groups tested, the predominant bacterium in the caecum was *E. coli* JNW14, and the bacterial numbers in the caecum reached $10^{10}$ cfu/g of caecal content (Table 2). The number of *E. coli* JNW14 in CBDL rats (2.56 SD 1.06 $\times 10^{10}$ cfu/g of caecal content) was significantly higher than that in the sham operation rats (1.43 SD 0.62 $\times 10^{10}$ cfu/g), indicating that an absence of bile acids in the intestinal tracts might allow *E. coli* to overgrow in the caecum. The numbers of viable *E. coli* JNW14 gradually decreased from 12 h after hepatectomy, although the reason for this is unclear.

The incidence of translocation of *E. coli* JNW14 and other bacteria to the MLNs, liver, lungs and spleen in all groups is shown in Table 3. Bacteria other than *E. coli* JNW14 were found in the organs of some of the rats in each group, except for the spleen in the sham operation group and the portal vein blood collected at 3 h after hepatectomy in group 3. However, the origin of these bacteria could not be identified.

*E. coli* JNW14 was translocated to the MLNs in all rats in each group, and the incidence of translocation to the MLNs was higher than that to the other organs. No

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Table 3. Incidence of bacterial translocation to various organs in antibiotic-treated rats given cultures of E. coli JNW14

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Culture</th>
<th>Total</th>
<th>JNW14</th>
<th>Culture</th>
<th>Total</th>
<th>JNW14</th>
<th>Culture</th>
<th>Total</th>
<th>JNW14</th>
<th>Culture</th>
<th>Total</th>
<th>JNW14</th>
<th>Culture</th>
<th>Total</th>
<th>JNW14</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sham (8)</td>
<td>E. coli</td>
<td>8 (100)</td>
<td>8 (100)</td>
<td>8 (100)</td>
<td>0 (0)</td>
<td>1 (13)</td>
<td>0 (0)</td>
<td>2 (25)</td>
<td>2 (25)</td>
<td>1 (13)</td>
<td>0 (0)</td>
<td>1 (13)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2. CBDL (7)</td>
<td>E. coli</td>
<td>7 (100)</td>
<td>7 (100)</td>
<td>7 (100)</td>
<td>2 (29)</td>
<td>1 (14)</td>
<td>0 (0)</td>
<td>2 (29)</td>
<td>2 (29)</td>
<td>2 (29)</td>
<td>2 (29)</td>
<td>2 (29)</td>
<td>2 (29)</td>
<td>0 (0)</td>
<td>1 (14)</td>
</tr>
<tr>
<td>3. CBDL + hepatectomy</td>
<td>E. coli</td>
<td>5 (63)</td>
<td>6 (75)</td>
<td>6 (75)</td>
<td>5 (63)</td>
<td>3 (38)</td>
<td>6 (75)</td>
<td>3 (38)</td>
<td>3 (38)</td>
<td>3 (38)</td>
<td>3 (38)</td>
<td>3 (38)</td>
<td>3 (38)</td>
<td>0 (0)</td>
<td>1 (14)</td>
</tr>
<tr>
<td>12 h</td>
<td>E. coli</td>
<td>5 (71)</td>
<td>6 (86)</td>
<td>7 (88)</td>
<td>4 (57)</td>
<td>4 (57)</td>
<td>7 (88)</td>
<td>4 (57)</td>
<td>4 (57)</td>
<td>7 (88)</td>
<td>4 (57)</td>
<td>4 (57)</td>
<td>7 (88)</td>
<td>4 (57)</td>
<td>4 (57)</td>
</tr>
<tr>
<td>24 h</td>
<td>E. coli</td>
<td>8 (100)</td>
<td>8 (100)</td>
<td>8 (100)</td>
<td>8 (100)</td>
<td>8 (100)</td>
<td>8 (100)</td>
<td>8 (100)</td>
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Samples were plated on antibiotic-containing selective medium, and incubated aerobically to detect E. coli JNW14, or on non-selective medium and incubated anaerobically (anaerobic culture) or aerobically (total) to detect any bacteria.

Significantly different from the sham operation group 1; p < 0.05.

Significantly different from the sham operation and CBDL groups 1 and 2; p < 0.05.

Discussion

Berg and Garlington [13] used the term ‘bacterial translocation’ to describe the passage of viable indigenous bacteria from the gastrointestinal tract to extra-intestinal sites such as the MLNs, liver, spleen, kidney,
peritoneal cavity and bloodstream under some stress conditions. They suggested that bacterial translocation is induced by three inter-related factors: (a) overgrowth of intestinal bacteria resulting from disruption of the ecological equilibrium, (b) host immunodeficiency and (c) increased permeability of the intestinal mucosal barrier [32]. Many investigators have tried to determine the conditions in which bacterial translocation is induced, by experimental studies and clinical surveys. However, the culture techniques used in most studies did not provide direct evidence that translocating bacteria are really derived from intestinal microbial flora. In the present study, various bacteria were found in the organs of the rats tested, but it is difficult to interpret the results due to the lack of the evidence of the origin of these bacteria, except for \( E. coli \) JNW14. Phenotypic or genotypic markers are required to determine the role of intestinal bacteria in bacterial translocation. The use of \( E. coli \) JNW14, which was resistant to bacitracin, neomycin and streptomycin, allowed the behaviour of intestinal bacteria to be monitored and bacterial translocation in rats that had undergone CBDL and CBDL plus hepatectomy to be demonstrated.

Berg [30] and Berg and Owens [33] demonstrated a direct relationship between bacterial overgrowth in the intestine and frequency of bacterial translocation to the MLNs: indigenous intestinal bacteria are continuously translocated when their population level is \( >10^4 \) cfu/g
of caecal contents. The present study also showed this relationship. The number of *E. coli* JNW14 was $>10^{10}$ cfu/g of caecal contents and translocation to the MLNs was observed in all rats tested. Moreover, in rats that had undergone CBDL plus hepatectomy, the number of translocated *E. coli* JNW14 in the MLNs decreased as its caecal population level decreased. These results suggest that special attention should be paid to intestinal overgrowth of certain bacterial strains during antibiotic treatment, to prevent the occurrence of infective complications.

In almost all of the rats in the sham operation and CBDL groups (1 and 2), translocated bacteria were restricted to the MLNs. On the other hand, in the CBDL plus hepatectomy group 3, *E. coli* JNW14 was found in other organs, such as the lungs, liver and spleen. These results indicate that (i) the first stage of bacterial translocation occurs in the MLNs and (ii) intestinal bacteria do not easily pass through the MLNs unless a high degree of surgical stress is added. These results are in good agreement with those reported by Wang *et al.* [34, 35], showing that the incidence of bacterial translocation to the MLNs was significantly higher than that to the portal vein. The absence of *E. coli* JNW14 in portal vein blood and the high frequency of bacterial translocation to the lungs (lungs $>$ liver $>$ spleen) indicate that the route from the MLNs to the lungs plays a important role in dissemination of translocated bacteria to other systemic organs. Even in the sham and CBDL groups (1 and 2), *E. coli* JNW14 was found in the lungs of several rats (Table 3). Based on these results, the major route of bacterial translocation appears to be lymphogenous and
the lymphatic-thoracic duct systemic circulation may play a major role in bacterial translocation. This would explain why the incidence of respiratory infection is high after hepatobiliary surgery. However, it is possible that bacteria translocated into the blood stream might be quickly sequestered and killed by the reticulo-endothelial system (RES) or that strain JNW14 might be more sensitive to the rat serum than other E. coli strains, explaining why E. coli JNW14 could not be detected in portal vein blood. The host immune system, especially cell-mediated immunity by macrophages and T cells, plays an important role in defence against bacterial translocation [32, 36]. Berg et al. [21] suggested that a combination of bacterial overgrowth in the intestine and host immunosuppression would synergically enhance the systemic spread of translocated bacteria from the MLNs to other organs. More extensive stress would allow the bacteria to flow out from the RES by suppressing the host immune system and make detection of the bacteria in the portal vein possible. Based on the findings described above, it is concluded that the degree of bacterial translocation to systemic organs depends on the number of viable cells translocated to the MLNs and the bactericidal activity of the MLNs.

This study also showed a significant relationship between the numbers of viable bacteria in the MLNs and the degree of translocation to other organs (Fig. 3). Bacterial overgrowth in the intestinal tract may be the most important factor in bacterial translocation to the MLNs, but several studies have demonstrated that histopathological changes such as villus oedema, dilated lymph vessels and inflammatory cell infiltration in a CBLD model are closely related to increased permeability of the intestinal mucosa [27, 28, 37, 38]. Furthermore, Wang et al. [24, 39] suggested that reduction in intestinal blood flow, diminished extraction of oxygen by the intestinal tissue, altered intestinal functions and morphology and intestinal paralysis induced by extensive liver resection contribute to attachment and overgrowth of enteric bacteria on the intestinal surface. In contrast, Schimpi et al. [40] reported that there was no macroscopic change in the jejunum and ileum in the same model and that histological alterations in the intestinal mucosa, such as changes in cell membrane structures and disruption of tight junction, occur at the subcellular level.

In summary, bacterial translocation was studied after extensive liver resection in jaundiced rats with the indigenous E. coli strain JNW14. This study revealed that the caecal population of bacteria and the viable bacterial numbers in the MLNs are important factors in assessing the degree of bacterial translocation. The high frequency of translocation to the lungs and absence of bacteria in the portal vein suggests that the intestinal bacteria would be translocated in the order of MLNs-(thoracic duct)-lung-liver and/or spleen. Further studies with the model presented here should provide useful information for understanding mechanisms of bacterial translocation after extensive liver resection in obstructive-jaundiced patients and for the development of effective therapy to prevent bacterial translocation.

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References


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