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Bacterial translocation and gut microflora in obstructive jaundice

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ABSTRACT

Bacterial translocation from the gut is implicated in the pathophysiology of complications associated with obstructive jaundice. Absence of intraluminal bile salts and their antiendotoxic effects may result in overgrowth of bacteria, promoting bacterial translocation. The large bowel is the largest source of gram negative bacteria but the small bowel is more permeable. This study investigated the effect of obstructive jaundice on bacterial translocation and on the indigenous luminal microflora at 3 sites in the gastrointestinal tract. Significant bacterial translocation was demonstrated following 7 d of bile duct ligation compared with control or sham operated groups. A qualitative disturbance of the caecal indigenous microflora was demonstrated in jaundiced rats. There was no 'site-to-site' variation in the indigenous microflora at the 3 intestinal sites studied. We conclude that experimental obstructive jaundice for 1 wk promotes bacterial translocation without significant quantitative disturbance of the intestinal microflora in the small intestinal or caecum.

Key words: Gastrointestinal tract; bile salts.

INTRODUCTION

Invasive diagnostic and therapeutic procedures in the presence of obstructive jaundice are associated with a high morbidity and mortality, primarily due to septic complications and renal impairment (Holman et al. 1979; Blamey et al. 1983; Dixon et al. 1983; Wait & Kahng, 1989). Systemic endotoxaemia has been implicated in the development of these sequelae (Wilkinson et al. 1976; Pain & Bailey, 1987) but the exact mechanism and pathophysiological sequence of events remain unclear. It is postulated that there are 2 contributing factors; one is increased bacterial translocation from the gastrointestinal tract across the gut mucosal barrier into the portal circulation (Koscar et al. 1969; Bailey, 1976), the other is impaired mononuclear phagocytic function which allows 'spillover' of endotoxin into the systemic circulation (Pain, 1987; Ding et al. 1992; Clements et al. 1993). Bacterial translocation is defined as the passage of viable indigenous bacteria from the gastrointestinal tract to normally sterile extraintestinal sites, such as the mesenteric lymph node (MLN) complex, spleen, liver, kidney, peritoneal cavity and bloodstream (Berg & Garlington, 1979). Factors which promote this process include disruption of the gut microecology, impairment of host immunity and physical injury of the gut mucosa (Deitch, 1990). Although the large bowel is the largest source of gram negative bacteria, the small bowel is known to be more permeable (Warshaw et al. 1977). Additionally, bile salts are known to inhibit the growth of intestinal bacteria (Floch et al. 1971; Williams et al. 1975), and may contribute to the regulation of the indigenous gut microflora. Absence of intraluminal bile salts may therefore allow bacterial overgrowth and, as bile salts are predominantly reabsorbed in the terminal ileum, this may be more pronounced in the small bowel. The aim of this study was to investigate the effect of obstructive jaundice on bacterial translocation and to assess the correlation with overgrowth of the indigenous microflora at 3 sites in the gastrointestinal tract, the jejunum, ileum and caecum.

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MATERIALS AND METHODS

Animals and experimental design

Adult female Wistar rats (250–300 g) were housed under constant temperature (22 °C) and humidity, with 12 h dark/light cycles and allowed standard laboratory animal chow (Robert Morton and Co. Ltd, Ballymena, UK) and water ad libitum throughout the experimental period. Rats were randomised to having no operation (controls) (n = 8), bile duct ligation (BDL) (n = 8), or sham operation (n = 9). After a period of 1 wk the animals were anaesthetised, portal and systemic blood was collected, and segments of bowel and the intra-abdominal solid organs were harvested for microbiological culture.

Operation

The method described by Lee (1972) was employed for bile duct ligation. Briefly, through a small upper midline abdominal incision, the common bile duct was identified and mobilised. It was then doubly ligated using 5–0 silk and divided. Sham operated animals had a similar incision followed by mobilisation of the common bile duct, without ligation or division. All abdominal incisions were closed in 2 layers using 4–0 chromic catgut. Each procedure was performed under general anaesthesia established using intramuscular Ketamine 10 mg/100 g (Parke-Davis Veterinary, Gwent, UK) and Xylazine 1.0 mg/100 g (Bayer UK Ltd, Bury St Edmunds, UK), observing strict asepsis.

Bacterial translocation

Seven days following operation, the rats were anaesthetised and laparotomy was performed under sterile conditions.

Portal and systemic blood culture. 3 ml samples of blood were obtained from both the portal vein and the inferior vena cava and cultured aerobically and anaerobically using the BacT/Alert (Organon Tecknika, Durham, NC, USA) (Thorpe et al. 1990). Blood cultures were continuously monitored for 7 d. Positive blood cultures were plated out on appropriate media and species identified by standard bacteriological techniques.

Homogenisation of organs. The mesenteric lymph node (MLN) complex, spleen and right lobe of liver were removed and placed in preweighed sterile glass bottles containing sterile prereduced brain-heart infusion. The bottles were reweighed and tissue homogenates were prepared in 2 ml brain–heart infusion using sterile mortars and pestles.

Preparation of bowel contents. 2 cm of bowel were harvested from the following sites: 5 cm distal to the gastric outlet (jejunum), 5 cm proximal to the caecum (ileum) and from the caecum itself. Each segment of bowel was placed in a preweighed sterile glass bottle containing sterile prereduced brain–heart infusion. The bottles were reweighed and transferred to an anaerobic cabinet (Concept 300 Anaerobic work station, Ruskinn Technology, UK) where the intraluminal contents were recovered. Serial dilutions ranging from 10^{-2} to 10^{-7} were prepared in $\frac{1}{4}$ strength Ringers lactate solution.

Quantitative and qualitative culture. 0.1 ml aliquots of each of the homogenates were cultured on blood agar, chocolate agar, McConkey agar, prereduced tryptone soya agar (TSA) and blood agar containing neomycin 75 μg/ml. Plates containing the latter 2 media were placed in an anaerobic cabinet. A further 0.2 ml aliquot was placed in cooked meat broth and plated out on the standard agar plates the following day. All plates were incubated at 37 °C. After the appropriate incubation periods, individual colonies were identified and quantified as colony-forming units (c.f.u.) per gram tissue using the formula:

$$\text{c.f.u. per gram tissue} = \frac{N \times D \times 2 \times 10}{W}$$

where N is the number of colonies on the plate, D the dilution inoculated on the plate, W the weight of specimen in grams, 2 for 2 ml brain–heart infusion and 10 for the 0.1 ml inoculum.

Statistical analysis

Nonparametric statistical analysis (Fisher's exact test and Mann Whitney U test) were employed throughout and statistical significance accepted at the 5% level.

RESULTS

There were no deaths in any group after 7 d. There was a significant increase in mean bilirubin in rats following bile duct ligation compared with the control groups: 288.8 μmol/l (BDL) vs 5.9 μmol/l (sham) vs 3.2 μmol/l (control) ($P < 0.001$, Mann Whitney U Test).

Bacterial translocation

There was no bacterial translocation either in the control or the sham operated groups, but bacterial translocation was demonstrated in 5 of the 8 rats following bile duct ligation ($P = 0.026$, Fisher's exact

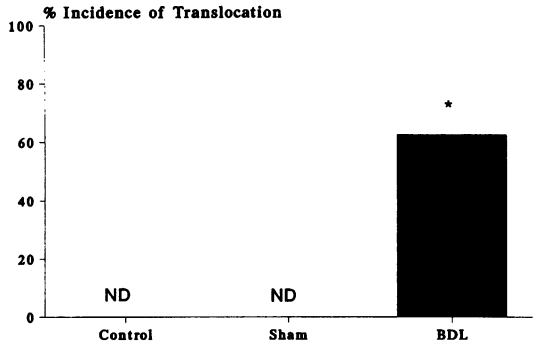

Fig. 1. Graph demonstrating incidence of bacterial translocation. ND, none detected; BDL, bile duct ligated. ($P = 0.026$, Fisher's exact test).

Table 1. *Percentage prevalence of gram negative bacilli in the caecum*

Organism	Control	Sham	BDL
E. coli	100	100	100
Pseudomonas	25	22	63
Pasteurella	13	11	75
Shigella	0	11	25
Proteus	0	0	13

BDL, bile duct ligation.

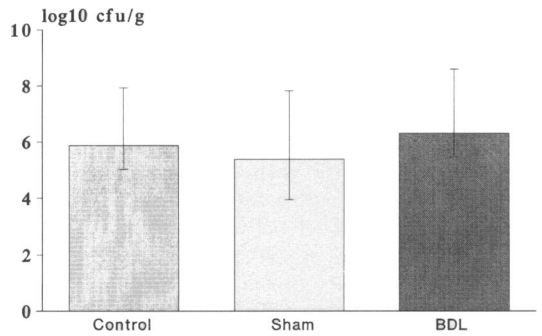

Fig. 2. Population levels of gram negative bacilli in the caecum. BDL, bile duct ligated.

test) (Fig. 1). In each of these animals bacteria were cultured from the mesenteric lymph node complex, and in 1 animal bacteria were also cultured from the liver and portal blood. The organism cultured in all cases was *Escherichia coli.*

Intestinal microflora

A broader spectrum of gram negative organisms was cultured from the caecum of jaundiced rats, reflecting a qualitative disturbance of the caecal indigenous microflora. There was an increased prevalence of pseudomonas, pasteurella, shigella and proteus in jaundiced rats compared with the control groups (Table 1), but none of these species translocated. The spectrum of organisms cultured from the small bowel segments was similar between the 3 groups studied.

There was no between 'site-to-site' variation in the indigenous microflora at the 3 intestinal sites studied (Fig. 2).

DISCUSSION

The gastrointestinal tract performs many functions in addition to the complex processes of digestion and selective absorption. It has important endocrine, immunological and metabolic functions and also acts as a defence barrier to prevent bacteria and endotoxin crossing the mucosa and invading systemic organs and tissues. Failure of intestinal barrier function resulting in the systemic spread of gut-associated bacteria has been termed bacterial translocation. Berg and Deitch have suggested that even though indigenous bacteria may be continuously translocating from the gastrointestinal tract, these bacteria are killed en route or in situ in lymphoid organs and are not normally cultured from the blood or intra-abdominal solid organs (Deitch, 1990; Berg, 1992). Bacterial translocation has been shown to occur under a variety of experimental insults such as bowel ischaemia (Deitch et al. 1990*a*), trauma (Deitch & Bridges, 1987), thermal injury (Maejima et al. 1984) and following the systemic administration of endotoxin (Deitch et al. 1987). Following numerous studies, Deitch and Berg have demonstrated that there are 3 mechanisms promoting bacterial translocation: (1) disruption of the ecological balance of the normal indigenous microflora, resulting in bacterial overgrowth of gram negative bacilli (Berg & Owens, 1979); (2) impairment of host immunity (Berg et al. 1988); and (3) physical injury of the gut mucosa (Morehouse et al. 1986).

Under normal circumstances, the indigenous bacteria have the ability to maintain communal stability, thereby contributing to the defence mechanism of the gut and preventing bacterial translocation. Bacterial antagonism is the term used to define a process whereby the resident microflora compete for nutrients and adhesion sites, and also produce antimicrobial factors which inhibit the overgrowth of an individual colony (Hentges, 1983). The indigenous bacteria also have the ability to prevent colonisation by non-indigenous or exogenous bacteria, and this has been termed colonisation resistance (Van der Waiij, 1987). It is hypothesised that disturbance of the indigenous microflora may result in loss of these 2 protective features, thereby increasing the likelihood of bacterial translocation.

Despite numerous studies concerning bacterial translocation, the anatomical site where translocation occurs remains unclear. Although the colon is the largest reservoir for gram negative organisms, the small intestine is known to be more permeable to macromolecules than the large bowel (Warshaw et al. 1977). Steffen & Berg (1983) demonstrated a correlation between the incidence of bacterial translocation to the mesenteric lymph node complex and caecal bacterial overgrowth in mice. Previous studies have demonstrated disruption of the caecal indigenous microflora in experimental extrahepatic biliary obstruction, and we were therefore interested to assess the microflora at different sites in the gastrointestinal tract for qualitative or quantitative changes and to correlate this with bacterial translocation. Deitch et al. (1990*b*) reported a 100-fold increase in the caecal population of gram negative aerobes in jaundiced mice in which translocation occurred compared with those which did not have viable bacteria in their mesenteric lymph nodes. Ding et al. (1994) also demonstrated significantly increased caecal population levels of *E. coli* and aerobes/microaerobes in rats following both 3 d and 7 d bile duct ligation. In 2 other studies with jaundiced rats, Ding et al. (1993*a*, *b*) have shown a tendency towards increased caecal populations of *E. coli* but the results did not reach statistical significance.

In this study, we have demonstrated significant bacterial translocation in rats following bile duct ligation compared with controls. These results concur with previous findings (Deitch et al. 1990*b*; Ding et al. 1993*a*, *b*, 1994; Reynolds et al. 1995). Assessment of the indigenous microflora in the jejunum and the ileum revealed no differences beween the 3 groups studied. However, a broader spectrum of gram negative aerobes was cultured from the caecum of jaundiced rats, reflecting a qualitative disturbance of the caecal indigenous microflora. Although increased caecal levels of gram negative aerobes occurred, this was not statistically significant, a similar observation to that of Ding et al. (1993*a*, *b*).

Absence of intraluminal bile may be responsible for this disturbance of the indigenous microflora. Bile salts are known to have detergent properties by forming unabsorbable micellar aggregates with the endotoxin molecule (Iwasaki & Tanikawa, 1981). They have also been shown to inhibit the growth of intestinal bacteria both in vitro and in vivo (Floch et al. 1971; Williams et al. 1975) and therefore may play an important role in the regulation of the indigenous gut microflora. Bile salts are predominantly reabsorbed in the terminal ileum and therefore very small quantities pass through to the large bowel. It would be expected therefore that absence of intraluminal bile salts may allow overgrowth of small bowel microflora and have less effect on the microflora of the large bowel. However, in this study we have demonstrated the caecum to be the site most susceptible to changes in the indigenous microflora, and that overgrowth of bacteria did not occur in the small intestine.

In summary, we have demonstrated bacterial translocation in an experimental model of biliary obstruction. Disturbance of the intestinal microflora may be partially responsible for this phenomenon, but other factors such as physical injury of the gut mucosa, impaired local or systemic immunity and systemic endotoxaemia may also be involved.

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