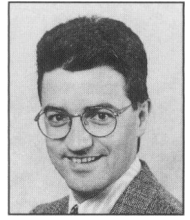# Gut Barrier Dysfunction in Obstructive Jaundice
THE PURCE LECTURE 1997

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INTRODUCTION

Invasive diagnostic and therapeutic procedures in patients with obstructive jaundice are associated with high morbidity and mortality, due to the development of complications, such as renal failure, sepsis, coagulation disorders and impaired wound healing.[1,2] The high incidence of these complications has prompted much research into the underlying pathophysiological mechanisms responsible for their development and also into various potential therapeutic strategies aimed at reducing their incidence. Proposed aetiological factors include the presence of potential toxic substances in the circulation, such as bilirubin and bile salts, overt or latent hypovolaemia, hypotension, and impaired nutritional status[3-5]. However, since Wardle and Wright demonstrated an association between the presence of endotoxaemia and renal failure in obstructive jaundice[6], endotoxin has been increasingly implicated in the pathophysiology of the complications seen in jaundiced patients.

It is currently postulated that there are two major contributing factors in the development of endotoxaemia in obstructive jaundice. One is impaired gastrointestinal barrier function allowing permeation of bacteria and endotoxins into the portal circulation and the lymphatic drainage system of the gut.[7,8] The other is impaired reticuloendothelial cell phagocytic function resulting in reduced clearance of bacteria and endotoxin[9,10], thereby allowing "spillover" of endotoxin into the systemic circulation with the subsequent development of systemic complications (Figure 1).
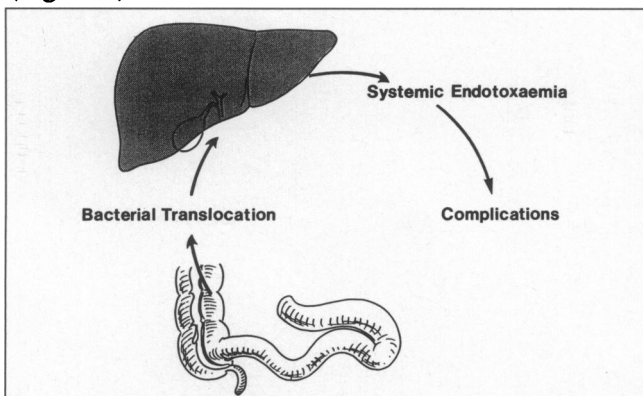

Figure 1

The intestine performs many functions, including the complex processes of digestion, selective absorption, secretion and immune modulation. In addition, it normally prevents bacteria contained within the lumen of the gut from escaping and invading systemic organs and tissues. When indigenous bacteria or endotoxin pass from the gut lumen to normally sterile extraintestinal sites, such as the mesenteric lymph nodes, liver, spleen, peritoneal cavity and bloodstream, the process is known as bacterial translocation and its occurrence implies gut barrier failure[11]. A significantly higher incidence of septic complications has been shown in patients who have evidence of bacterial translocation at the time of laparotomy (28%) compared to those who do not (11%).[12] The integrity of the mucosal barrier can also be assessed by measuring intestinal permeability. Increased permeability implies functional impairment of the intestinal mucosal barrier and has been shown in burn patients to be a significant risk factor for the development of sepsis.[13,14]

The aim of these studies was to investigate gut barrier function in an experimental model of biliary obstruction and in jaundiced patients. The incidence of bacterial translocation was examined following bile duct ligation in rats, and factors known to promote this phenomenon were studied. Changes in intestinal permeability were also determined in this animal model and in patients with obstructive jaundice. In addition, the effect of internal biliary drainage on intestinal permeability was assessed in the clinical setting.

ANIMAL STUDIES

Adult female Wistar rats (250 - 300g) were used in the following series of studies. Experimental extrahepatic obstructive jaundice was produced using the method described by Lee[15]. Significantly raised bilirubin concentrations were recorded in all animals following bile duct ligation.

(1) Bacteriological Study

Rats were randomised to having no operation (n=14),

bile duct ligation (BDL) (n=16) or sham operation (n=16). After a period of one week, the animals were anaesthetised and laparotomy was performed under sterile conditions. Portal blood was collected, and segments of the intra-abdominal solid organs and caecum were harvested. Samples were cultured aerobically and anaerobically at 37°C. After appropriate incubation periods, individual colonies were identified by standard bacteriological techniques.[16] Bile duct ligation resulted in a significantly increased incidence of bacterial translocation compared with animals having no operation or sham operation (68.5% BDL vs 6.3% Sham vs 0% No operation, P < 0.01, Fisher's Exact test). Translocation was predominantly to the mesenteric lymph nodes and the organism most commonly cultured was Escherichia coli.

A broader spectrum of gram negative organisms was cultured from the caecum of jaundiced rats compared with the control groups, with an increased prevalence of pseudomonas, pasteurella, shigella and proteus. In addition to this qualitative disturbance, a quantitative disturbance of the indigenous caecal microflora was also demonstrated following bile duct ligation. A significant increase in the caecal gram negative aerobic population was shown as evidence of bacterial overgrowth.

(2) Morphological Study

Rats were assigned to one of three groups: no operation (n=8), bile duct ligation (n=11) or sham operation (n=10). One week following intervention, the animals were anaesthetised and laparotomy was performed. Segments of bowel were harvested from the following sites: 5 cm distal to the gastric outlet (jejunum), 5 cm proximal to the caecum (ileum), the caecum itself and the transverse colon. The bowel was opened along its length and immediately fixed in formalin. After fixation, segments were embedded in paraffin wax and 5µm sections were then cut and stained with haematoxylin and eosin. Histological evaluation was performed by an independent pathologist. Well-orientated sections showing good preservation of structure and cytological detail were selected for morphometric assessment using a computerised image analysis system.

There was no obvious breach or gross ulceration of the mucosa in any of the animals studied. Morphometric assessment showed no difference in mucosal measurements in the jejunum, caecum or colon. However, significant morphological changes were demonstrated in the terminal ileum of jaundiced rats

compared with the control groups of animals (Table 1). The reduction of total mucosal thickness observed in this study largely reflected a significant decrease in villus height.

TABLE 1

Ideal measurements (pm)			
	Mucosal thickness	Villus height	Crypt depth
Control	744 (95)	559 (79)	183 (19)
Sham	731 (27)	515 (18)	193 (11)
BDL	650 (23) *	451 (20) *	180 (8)

Morphometric measurements of ideal mucosa. Results are expressed as mean (SEM). * P < 0.02, Mann-Whitney U test.

(3) Intestinal Permeability Study.

Rats were randomised to undergo bile duct ligation (n=12) or sham operation (n=12). Intestinal permeability was measured by calculating the percentage change in the 24 hour urinary recovery of orally administered radiolabelled polyethylene glycol 4000 (^{14}C PEG 4000) given 1 week prior to operation and 1 week following operation with each animal acting as its own control[17]. PEG 4000 was used as it is a macromolecule whose size mimics that of antigenic substances, such as endotoxin.

There was a significant increase in intestinal permeability in bile duct ligated animals compared with sham operated controls (+66.2% BDL vs -11.6% Sham, P<0.01, Mann Whitney U test) (Figure 2).
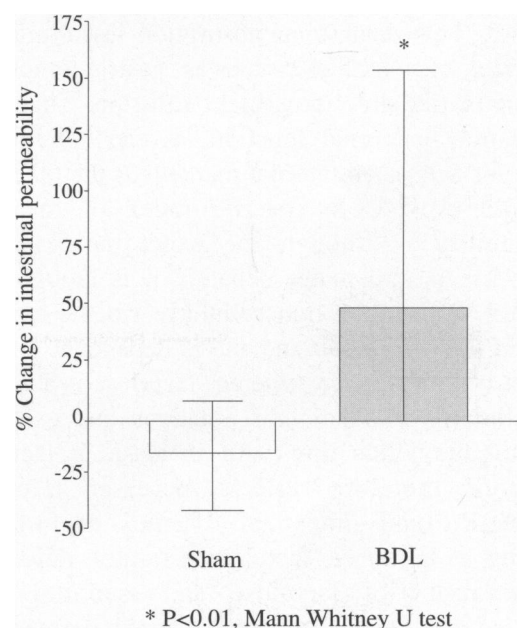

Figure 2

* P<0.01, Mann Whitney U test

CLINICAL STUDY

A prospective study was performed on 45 consecutive patients with obstructive jaundice (bilirubin > 100 μmol/1) undergoing internal biliary drainage as a definitive therapeutic procedure by surgical (n=11), endoscopic (n=16) or percutaneous (n=18) means. Of those patients who had surgical intervention, five patients had choledocholithiasis and underwent open cholecystectomy with exploration of the common bile duct, and six had malignant biliary obstruction. Of the later group, two had a Whipple procedure, one had a localised resection of a periampullary tumour and three had biliary bypass procedures performed without resection.

Ten nonjaundiced patients undergoing laparotomy (n=9) or diagnostic endoscopic retrograde cholangiopancreatography (ERCP)(n=1) were also studied to act as control patients. All patients received 1.5g cefuroxime immediately prior to intervention. Systemic or oral antibiotics were not administered routinely following intervention, but were administered if clinically indicated. Each patient was studied on the day prior to therapeutic intervention and on days 1, 7 and 28 following their procedure. Routine liver function tests were performed and intestinal permeability was measured. In addition, intestinal permeability was assessed in 11 healthy volunteers to obtain a reference value for our test of intestinal permeability which would reflect normal gut barrier function.

Intestinal Permeability

Intestinal permeability was assessed by measuring the urinary excretion of orally administered lactulose and mannitol. This dual sugar absorption test eliminates the effect of variables, such as gastric emptying, intestinal transit, intestinal dilution, bacterial degradation and renal function, as each of the test molecules acts as an internal marker for the other and therefore provides a specific index of intestinal permeability.[18] Although the exact pathways the probes use is still under debate,[19] it is thought that mannitol is absorbed transcellularly via the aqueous pores in the cell membrane and is dependant on total absorptive surface area, whereas lactulose is absorbed paracellularly, via the tight junctions and extrusion zones at the villus tips.[20] An increase in lactulose absorption therefore reflects increased intestinal "leakiness" and intestinal barrier dysfunction, resulting in a raised lactulose/mannitol ratio. The concentrations of lactulose and mannitol were measured enzymatically and intestinal permeability was expressed as the lactulose / mannitol ratio [17]

The mean serum bilirubin in the jaundiced group prior to therapeutic intervention was significantly higher than in the control group of patients (235 μmol/l vs 7.5 μmol/1, P<0.0001, Mann Whitney U test). Following therapeutic intervention, the mean serum bilirubin in the jaundiced group of patients was progressively lower at each of the time points studied (182 ,μmol/1 Day +1 vs 73 μmol/1 Day +7 vs 21 μmol/l Day +28).

The median [interquartile range (i.q.r.)] lactulose / mannitol permeability index in 11 normal healthy volunteers [0.019 (0.012 - 0.030)] was not significantly different from the control group of nonjaundiced patients [0.015 (0.011 - 0.021)] (P = 0.377, Mann Whitney U test). However, the median (i.q.r.) lactulose / mannitol ratio for patients with unrelieved obstructive jaundice [0.033 (0.021 - 0.046)] was significantly higher than that of the control group of patients studied preoperatively (P<0.0001, Mann Whitney U test). Twenty eight days following intervention, the lactulose / mannitol ratio in patients who had presented with obstructive jaundice had returned to normal and was significantly less than on the day before internal biliary drainage (P < 0.05, Wilcoxon's Signed Rank test) (Figure 3).
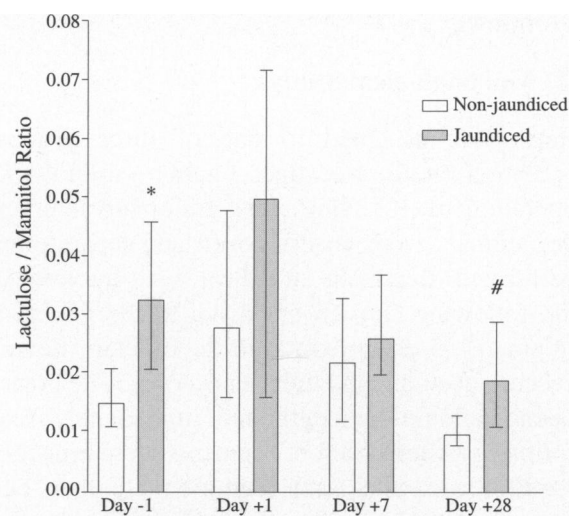

Figure 3
* P < 0.0001, vs Non-jaundiced, Mann Whitney U test
# P < 0.05, vs Day -1, Wilcoxon's Signed Rank test

DISCUSSION

These studies have demonstrated evidence of impaired intestinal barrier function in obstructive jaundice. Significant bacterial translocation and significantly increased intestinal permeability were shown following bile duct ligation in an animal model. In addition, significantly increased intestinal permeability was demonstrated in jaundiced patients and this impairment of gut barrier function was shown

to be reversed by returning bile to the gastrointestinal tract.

Bacterial translocation following bile duct ligation has been reported by others[21-24], However, the mechanisms by which bacteria escape from the intestinal lumen remain to be clearly defined. Deitch, has proposed three factors which may promote this process, namely, disruption of the indigenous gut microecology, physical injury of the gut mucosa and impairment of host immunity.[25] Previously, our group has demonstrated impaired reticuloendothelial cell function in jaundiced rats.[10] In these present studies, we have demonstrated a qualitative and quantitative alteration of the indigenous caecal microflora following bile duct ligation. Bacterial overgrowth of the gram negative aerobes was associated with significant bacterial translocation, predominantly to the mesenteric lymph nodes. In addition, we have demonstrated morphometric evidence of intestinal mucosal injury in jaundiced rats.

Absence of intraluminal bile may be responsible for these findings. Bile salts are known to inhibit the growth of intestinal bacteria both in vitro and in vivo[26,27] and, therefore, their absence may allow overgrowth of certain species of bacteria and thus promote the likelihood of that species translocating from the gastrointestinal tract. In addition, Iwasaki and Tanikawa reported that bile salts had anti-endotoxin effects by breaking down the endotoxin molecule into subunits and forming unabsorbable micellar aggregates.[28] Therefore, loss of the emulsifying properties of intraluminal bile salts could result in a larger pool of endotoxin available for translocation into the portal circulation. Bile is also known to promote cell proliferation and has a trophic effect upon intestinal mucosa.[29,31] Hence, absence of intraluminal bile may also contribute to the morphological changes demonstrated following bile duct ligation.

To date, there has been no evaluation of intestinal permeability in obstructive jaundice. The finding of significantly increased intestinal permeability in the experimental study was confirmed in the clinical study and this provides the first reported evidence of gut barrier dysfunction in jaundiced patients.

In the clinical study, intestinal permeability was increased on the first postoperative day in both jaundiced and nonjaundiced patients. This increase was more marked in patients who underwent surgical intervention than in those who had non-operative biliary decompression, and this may reflect a further

endotoxin challenge to intestinal barrier function through exposure of the peritoneal cavity to endotoxin in the air at laparotomy[40]. By the seventh postoperative day the lactulose / mannitol ratio had returned to preoperative levels. More importantly, in patients with jaundice, the intestinal permeability index continued to fall and after 28 days of internal biliary drainage had returned to normal.

In conclusion, these studies demonstrate evidence of impaired intestinal barrier function following experimental biliary obstruction with significant bacterial translocation and increased intestinal permeability. There was also significantly increased intestinal permeability in jaundiced patients which returned to normal after 28 days of internal biliary drainage. This suggests that return of bile to the gastrointestinal tract may improve intestinal barrier function and reduce the incidence of gut-derived sepsis or other complications associated with endotoxaemia in obstructive jaundice.

ACKNOWLEDGEMENT

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