Biliary decompression promotes Kupffer cell recovery in obstructive jaundice

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Abstract

Background—Jaundiced patients undergoing surgical procedures have an increased risk of Gram negative sepsis with potential morbidity and mortality. Depressed Kupffer cell clearance capacity (KCCC) predisposes jaundiced patients to endotoxaemia and its sequelae. Biliary decompression remains the main therapeutic strategy in obstructive jaundice.

Aims—This study investigates the efficacy of internal (ID) and external biliary drainage (ED) on KCCC in an experimental model of extrahepatic biliary obstruction.

Methods—Adult male Wistar rats (250–300 g) were assigned to one of six groups: sham operated, where the bile duct was mobilised but not divided; bile duct ligation (BDL) for three weeks, and sham operated or BDL for three weeks followed by a second laparotomy and further 21 days of ID or ED, by way of choledochoduodenostomy or choledochovesical fistula respectively. KCCC was measured using an isolated hepatic perfusion technique with FITC labelled latex particles (0.75 μ) as the test probe. Plasma was assayed for bilirubin, endotoxin, and anticore glycolipid antibody (ACGA) concentrations.

Results—Jaundiced rats had reduced KCCC ($p < 0.001$), increased concentrations of ACGA ($p < 0.001$), and endotoxin ($p < 0.001$) compared with controls. Biliary drainage for three weeks produced a recovery in KCCC and normalisation of endotoxin and ACGA concentrations, however, external drainage was less effective than ID ($p < 0.01$).

Conclusions—These data support the hypothesis that endotoxaemia and its mediated effects are integral in the pathophysiology of jaundice. Furthermore, a short period of internal biliary drainage is a useful therapeutic strategy in restoring Kupffer cell function and negating systemic endotoxaemia and consequent complications in biliary obstruction.

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and wound breakdown are frequently recognised, however, sepsis accounts for the large proportion of complications and is reported in as many as 72% of patients postoperatively.^{2,3}

Endotoxin is a constituent of the cell wall of Gram negative bacteria and the lipid A moiety, a highly conserved inner core structure, is a powerful immunogenic molecule with potential to cause a vast array of deleterious physiological effects. There is overwhelming clinical and experimental evidence demonstrating the occurrence of systemic endotoxaemia in obstructive jaundice and many authors feel that endotoxin, either directly or indirectly through an array of mediated effects has an integral role in the pathophysiology of biliary obstruction.⁴ There are two hypotheses for the mechanism of systemic endotoxaemia (Fig 1). Absence of intraluminal bile salts promote bacterial overgrowth and translocation of bacteria and endotoxin from the large intestine into the portal circulation.⁵ Depressed Kupffer cell clearance capacity in the liver permits spillover of endotoxin into the systemic circulation.⁶ Several authors have shown that mononuclear phagocytic function is depressed in obstructive jaundice, however, none have concentrated on the specific role of the Kupffer cell population, which have a central role in the development of endotoxaemia in biliary obstruction. In a previous study using an in situ hepatic perfusion technique we have shown that Kupffer cell clearance capacity (KCCC) is depressed in an experimental model of extrahepatic biliary obstruction.⁷

Although improvements in anaesthesia, perioperative antimicrobial prophylaxis with technological developments in endoscopy, and interventional radiology have served to reduce perioperative morbidity and mortality,^{8–10} the incidence of septic complications in jaundiced patients remains unacceptably high.^{11–15}

Biliary decompression remains the main therapeutic strategy in patients with obstructive jaundice, although there is little scientific evidence to support its use. In this study we examined the effects of biliary decompression, by internal or external means,¹⁶ on KCCC, in an experimental model of extrahepatic biliary obstruction.

Methods

PERFUSION TECHNIQUE

We used a novel in situ isolated hepatic perfusion technique to specifically measure KCCC.¹⁷ The liver was surgically isolated as described by Hems *et al.*¹⁸ Briefly, 100 IU of heparin was given initially via the saphenous

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Extrahepatic obstructive jaundice is a common surgical condition. Patients suffering from this disease have an increased susceptibility to complications and death (20–30%).¹ Renal failure, gastrointestinal haemorrhage, coagulopathy,

Figure 1: Diagrammatic representation of our hypothesis for the mechanism of systemic endotoxaemia. This outlines the role of the gut-liver axis in the pathophysiology of endotoxaemia in extrahepatic biliary obstruction.

vein and the liver isolated with 17G polystyrene cannulae placed in the portal and supradiaphragmatic vena cava via the right atrium. The hepatic artery was clipped, the infradiaphragmatic vena cava was ligated, and the liver perfused in an antegrade fashion through the portal vein (Fig 2). The perfusion medium used was haemoglobin free Krebs-Henseleit bicarbonate buffer supplemented with 0.5% glucose and 0.5% albumin. The test probe was fluorescein isothiocyanate labelled latex particles, 0.75 μm in diameter with a coefficient of variation for diameter and fluorescence of less than 5%, at a concentration of $10^5/\text{ml}$, which was the concentration whereby Kupffer cell clearance was maximal, observing zero order kinetics (Fluoresbrite particles, Polysciences, Northampton, England). Perfusate was oxygenated and heated (37°C)

to a physiological state in a 'glass lung' (Peter Brooks, Oxford University, Oxford, England) and delivered at a constant flow rate of 25 ml/min. The liver was initially perfused with buffer alone for 10 minutes to permit acclimatisation of the preparation. Subsequently, buffer containing latex particles was perfused for a further 10 minutes and 1 ml samples of effluent perfusate collected from the hepatic veins at one minute intervals.

Fluorescent particles appeared in the effluent perfusate within seconds and reached a steady state within two to three minutes. The mean value was taken for 10 readings throughout the period of the perfusion and the coefficient of variation ranged from 2.5%–8.75%. Transmission electron microscopy confirmed almost exclusive ingestion of particles by the Kupffer cell population (Fig 3). The fluorescence of the influent and effluent perfusate was measured (emission 540 nm/excitation 464 nm) and clearance calculated from the difference in the two values. KCCC was expressed as a percentage of the influent perfusate.

Viability criteria were adjudged during and at the end of the experiment. Successful perfusion was based on the appraisal of the gross appearance of the liver, where there was uniform blanching of the liver substance with all lobes having a homogeneous appearance, unobstructed perfusate flow at a constant pressure of 10 cm H_2O , and maintenance of the perfusate pH (7.2–7.4). The effluent perfusate is analysed to ensure that potassium concentrations and transaminase (AST, ALT, and LDH) concentrations remain within normal limits ($<5 \text{ mmol/l}$ and $<20 \text{ IU/l}$ greater than serum concentrations, respectively). After perfusion the viability was assessed using both light and electron microscopy to exclude hepatocyte swelling and vacuolation, loss of microvilli, and swelling of mitochondria. The perfusion characteristics of the livers were the same in all groups studied.

ANIMAL MODELS (FIG 4)

Bile duct ligation

Animals were given an anaesthetic cocktail of 0.1 ml of Hypnorm (fentanyl citrate, 0.315 mg/ml; fluanisone, 10 mg/ml; Janssen Pharmaceutical, Oxford, England), and 0.25 mg of diazepam (Roche Products, Welwyn Garden City, England). Through a 1 cm upper midline the common bile duct was mobilised, doubly ligated with 5/0 silk at the porta hepatis and then divided.

Choledochoduodenostomy (ID) and choledochovesical fistula (ED)

The choledochoduodenostomy was performed using a modification of the technique described by Ryan *et al* in 1977.¹⁹ The method used was that described by Diamond,²⁰ whereby at the initial bile duct ligation a silastic cannula (Dow Corning, Reading, England) was introduced into the proximal common bile duct and secured using silk ties placed around

Figure 2: Diagrammatic representation of the components constituting the isolated organ perfusion system. The important component that marries the peristaltic pump, oxygen source, and reservoir of influent perfusate is the multibulb oxygenator (glass lung).

the common duct. The lower end of the cannula is kinked and ligated to cause biliary obstruction. The reversal operation is then simply a matter of dividing the ligature and inserting the distal end of the cannula into the duodenum through a purse string suture. The choledochovesical fistula was initially described by Burke *et al* in 1977²¹ and subsequently the technique was improved by Diamond and Rowlands in 1990.²² At the initial operation a silastic cannula is inserted into the common bile duct and the lower end introduced into the dome of the urinary bladder through a silk purse string suture. The middle portion of the cannula is kinked and ligated to produce biliary obstruction. At the reversal procedure the ligature is released permitting sterile external decompression of the biliary system via the bladder. This model has obvious advantages over other methods of external biliary drainage, namely the decreased risk of exogenous infection and inadvertent dislodgement of the cannula. Postcholestatic cholestasis occurs in the immediate period after ligature release in the CDVF group and must be replaced by daily subcutaneous injection of normal saline for five days after reversal.

ANIMAL GROUPS

Six groups of adult male Wistar rats (250–300 g) ($n=10$ per group) from our breeding colony were studied. Sham operated, where the bile duct was mobilised but not divided, bile duct ligation and division (BDL) for three weeks, and sham operated or BDL for three weeks followed by a second laparotomy after 21 days when either CDVF or CDD was performed to permit ED and ID respectively for a

further 21 day period. The animals were fed standard chow with water *ad libitum*.

ASSAYS

Prior to hepatic perfusion blood samples were collected aseptically from the inferior vena cava and immediately placed in heparinised vials on ice and subsequently centrifuged at 4°C. Plasma samples were aliquoted and stored at –70°C for subsequent assay. Endotoxin concentrations were assayed using the quantitative Limulus Lysate chromogenic assay (Quadrantech, Epsom, England) and expressed in picograms per millilitre. Anticore glycolipid antibody – the relative concentration of antibodies to the core region of circulating endotoxin – were measured using an enzyme linked immunosorbent assay technique that used microtitre plates coated with a cocktail of

Figure 3: Transmission electron micrograph of a typical hepatic sinusoid after perfusion with latex particles in a normal rat. The Kupffer cell (KC) nucleus is surrounded by latex particle (arrowed). The hepatocytes (H) are in close apposition to the sinusoidal cells.

four core highly conserved inner core glycolipid structures complexed with polymyxin B sulphate as described by Scott and Barclay.²³ Prediluted samples were incubated with the solid phase, and bound rat IgG was detected using a specific antirat IgG-peroxidase conjugate (Serotec, Oxford, England). The results are expressed as a percentage of the mean value obtained from a bank of normal control rats. Bilirubin concentrations were assayed using a standard technique, and results expressed in micromoles per litre.

Figure 4: Diagrammatic representation of the animal models used in the experiment. This cartoon outlines the method of internal and external biliary drainage used in our laboratory.

Summary of the results of Kupffer cell clearance capacity in jaundiced and rats treated with internal and external biliary decompression

Model	Bilirubin (μmol/l)	ACGA (% control)	Endotoxin (pg/ml)	KCCC (%)
BDL	168 (12.3)*	340.3 (60)*	100 (51.1)*	14.6 (1.8)*
Sham	1.2 (0.3)	120.2 (8.5)	7.1 (2.5)	38.5 (1.4)
S/ED	2.3 (1.1)	108 (15.6)	2.9 (1.7)	34.4 (2)
S/ID	2.7 (0.8)	140 (16.5)	3.7 (2.5)	40.1 (3.4)
B/ED	3.3 (1.5)	110.6 (7.2)	3.7 (2.6)	33.9 (1.9)†
B/ID	2.7 (0.9)	115.4 (10.9)	7.5 (6.2)	42.9 (1.6)

Data are mean (SEM). *p<0.001, †p<0.01. BDL=bile duct ligation for three weeks; sham=sham operation for three weeks; S/ED=sham operation (3/52) followed by choledochovesical fistula (3/52); S/ID=sham operation (3/52) followed by choledochoduodenostomy (3/52); B/ED=BDL (3/52) followed by choledochovesical fistula (3/52); B/ID=BDL (3/52) followed by choledochoduodenostomy (3/52); ACGA=anticore glycolipid antibody; KCCC=Kupffer cell clearance capacity.

Figure 5: Diagrammatic representation of the results of Kupffer cell clearance in experimental biliary obstruction and after internal and external biliary drainage.

Both these methods were used to increase our sensitivity for systemic endotoxin detection. The LAL assay serves as the gold standard method for endotoxin measurement, however, this technique has been plagued with a variety of physical and technical problems, which have limited its usefulness. The EndoCab assay overcomes many of the problems associated with direct measurement of plasma endotoxin.

STATISTICAL ANALYSIS

Statistical analyses were performed using the Kruskal-Wallis analysis of variance, Mann-Whitney U test, and the Spearman rank correlation coefficient. Differences were considered significant at the 5% level (p<0.05). Results are expressed as mean (SEM). Calculations were performed using Arcus Pro statistics software (Iain Buchan, Oxford, England).

Results

The Table summarises the results of the experiment. Mortality was minimal over the course of the experiment. Four rats died: one from the BDL group, one from the sham/ED group, and two from the BDL/ED group. The bile duct ligated rats were clinically jaundiced, thin but with similar energy levels to control animals. The bile duct ligated rats lost more weight than the sham operated animals but by two weeks postoperatively were starting to recoup the loss and gained weight at a similar rate to control rats. Bile duct ligated rats had pronounced dilatation of the proximal bile duct with hepatosplenomegaly. The wet liver to body weight ratio was significantly increased in the jaundiced rats (BDL3 0.0584 (0.00135), sham3 0.0386 (0.0006), p<0.0001 Mann-Whitney test). After biliary decompression both the internally and externally drained groups lost weight initially but by the seventh postoperative day were starting to gain weight at a similar rate to controls. Jaundice was visibly diminishing by the seventh day after reversal and at death on day 21 had returned to normal concentrations along with transaminase and albumin values. Liver to body weight ratio had returned to normal at termination of the experiment (BDL/ID 0.041+0.0014, BDL/ED 0.040+0.0019).

Kupffer cell clearance capacity was significantly reduced in rats jaundiced for three weeks compared with sham operated animals (p<0.001) (Fig 5). There was strong evidence of systemic endotoxaemia in the bile duct ligated rats. This was reflected in a significant increase in endotoxin (p<0.001) (Fig 6) and anticore glycolipid antibody (p<0.001) concentrations (Fig 7).

After a three week period of biliary decompression by both external and internal means KCCC had returned to normal (Fig 5). It was evident that rats undergoing internal biliary drainage had a significantly increased KCCC compared with rats treated by external drainage (p<0.01). External and internal biliary drainage were equally effective in negating systemic endotoxaemia (Figs 6 and 7).

Figure 6: Diagrammatic representation of the endotoxin concentrations in jaundiced rats and after internal and external biliary drainage for three weeks.

Endotoxin concentrations correlated with ACGA concentrations ($r=0.74$, $p<0.0001$). Both these parameters correlated inversely with KCCC ($r=0.61$, $p<0.0001$).

Discussion

Jaundiced patients undergoing invasive diagnostic and therapeutic procedures have an increased risk of complications and death.^{24–25} Gram negative sepsis constitutes the bulk of the morbidity and the mortality, however, disturbance in renal function,²⁶ coagulation,²⁷ and wound healing²⁸ are also inherent problems in this patient population. Experimental and clinical studies have identified several aetiological factors including hypotension, impaired nutritional status, depressed immune function, hepatic parenchymal dysfunction, and the toxic effects of bilirubin and bile acids. Wardle and Wright²⁹ in 1970, were the first to show an association between systemic endotoxaemia and renal dysfunction in jaundiced patients and since then many authors have concurred with these findings.^{30–32} With biochemical advances in methods for endotoxin detection³³ there is now universal agreement that systemic endotoxaemia is integral in the pathophysiology of obstructive jaundice.

Figure 7: Diagrammatic representation of the anticore glycolipid concentrations in jaundiced rats and after internal and external biliary drainage for three weeks.

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 over 85% of the cells of the mononuclear phagocytic system. They are strategically situated at the confluence of portal venous drainage and bear the major responsibility for sequestering and eliminating endotoxins delivered from the gut in the portal circulation. The functional heterogeneity of the various subpopulations comprising the mononuclear phagocytic system^{34–35} and their key anatomical situation reinforces the necessity for specific study of the Kupffer cell population in biliary obstruction.

Using a variety of methods to assess vascular clearance, several authors have shown that the mononuclear phagocytic system phagocytic capacity is depressed in biliary obstruction and restored after biliary decompression.^{36–39} An array of different test substances, namely, synthetic dyes, colloidal carbon, microaggregated albumin, and radiolabelled bacteria have been studied. Vascular clearance of particulate matter is a complex process dependent upon its concentration, blood flow, and specific physiochemical properties.^{40–41} We have applied an isolated hepatic perfusion technique to measure directly and continuously Kupffer cell clearance capacity. This system has certain advantages in that Kupffer cell function can be assessed in the absence of circulating blood and potentially influential serum factors. Flow to the isolated organ preparation can be carefully controlled and the liver can be studied as a syncytium in the intact physiological state.

The initial uptake of endotoxin by the liver is mediated by the Kupffer cell,⁴² and subsequently a modified form of lipopolysaccharide is transferred to the hepatocytes, presumably to be further detoxified.⁴³ A 80 kDa endotoxin binding protein has been identified on peritoneal macrophages and other monocyte cells,⁴⁴ however, this protein has not been identified in Kupffer cells. Most studies would indicate that endotoxin uptake in Kupffer cells is not receptor mediated. Unlike other monocytes Kupffer cells take up endotoxin by non-specific absorptive pinocytosis.⁴⁵ For this reason we used FITC-labelled latex particles in the perfusion system to measure Kupffer cell clearance. We have previously used this system to assess the effects of experimental biliary obstruction on KCCC and have shown that rats jaundiced for greater than two weeks have significantly impaired Kupffer cell clearance.⁷

The aetiology of Kupffer cell dysfunction in jaundice is still unclear. Potential causative factors include a change in cell membranes, cellular function, and Kupffer cell morphology induced by the detergent properties of bile salts.⁴⁶ This would not explain why mononuclear phagocytic system function is impaired in parenchymal disease where systemic bile salt concentrations are normal. Decreased concentrations of opsonins, such as fibronectin have also been implicated but results to date have been equivocal. Portal hypertension occurs after bile duct ligation and several authors have implied that decreased phagocytosis may be

the result of depressed hepatic sinusoidal perfusion. In contrast, others have shown no change in sinusoidal blood flow in the canine bile duct ligation model and jaundiced patients.^{38 47} Little is known of events at a microvascular level within the liver. We have shown in an image analysis study of Kupffer cells in the jaundiced rat that despite an increase in Kupffer cell size and numbers with excessive lysosomal bodies the sinusoidal space is massively compromised, which may effect uptake of particles.⁴⁸ We have also shown that there is a decrease in major histocompatibility complex classic antigen expression on mononuclear cells in the bile duct ligated rat⁴⁹ suggesting decreased activation of these antigen presenting cells. Despite the lack of information on the exact pathophysiological mechanism of depressed phagocytic capacity in jaundice, biliary decompression remains the main therapeutic strategy used in jaundiced patients. There is clinical and experimental evidence that biliary decompression results in global improvement in host immune function. There is still some disagreement on the period of decompression required to permit immunological recovery of the various cellular subpopulations constituting the host immune system. We have shown that three weeks of biliary decompression results in normalisation of Kupffer cell clearance capacity with complete negation of systemic endotoxaemia. Most experimental and clinical evidence points to a recovery period of at least weeks and possibly months for full immunological recovery^{37 39 50-52} although more recently Ding *et al* have shown that hepatic clearance of radiolabelled *Escherichia coli* has returned to normal within days of internal biliary decompression,³⁸ although the rats under study were subjected to a shorter period of bile duct ligation – that is, 14 days.

We concur with the findings of Gouma *et al* that internal biliary drainage is a better option than external drainage.⁵³ The implication of this finding highlights the importance of re-establishing bile flow to the gastrointestinal tract. It is recognised that biliary obstruction promotes bacterial translocation and this is related in part, to the overgrowth of Gram negative aerobic organisms, which occurs when the emulsifying action of bile salts are absent from the gut lumen.^{54 55} It is plausible that bacterial translocation initiates the influx of endotoxin into the portal circulation, which activates the Kupffer cell population to secrete proinflammatory cytokines locally. In high concentrations these cytokines are toxic to Kupffer cells and hepatocytes. They may also cause microcirculatory disturbances that will depress delivery of endotoxins to the Kupffer cells. Greve *et al* in an eloquent study showed the immunosuppressant effects of endotoxin on cellular immune function.³¹ We are presently investigating the primary role of endotoxin in Kupffer cell dysfunction using germ free animals. In summary this study confirms the therapeutic potential of internal biliary decompression in restoring Kupffer cell phagocytic function and elimination of systemic endotoxaemia with its consequent

secretory sequelae. Although decompression of the biliary tree is important in normalising hydrostatic pressures within the biliary tree, it is important to complement this with restoration of gastrointestinal bile flow.

The pathophysiology of complications occurring in biliary obstruction is complex and undoubtedly multifactorial. To date there is little information available in published reports on the role of cytokines in biliary obstruction. Research should be aimed at elucidating the effects of endotoxaemia on Kupffer cell secretory capacity and investigating potential nutritional, pharmacological and immunological strategies of augmenting Kupffer cell function, and promoting gastrointestinal mucosal integrity.

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