Br. J. Surg. 1986, Vol. 73, October, 775–778

A meeting of The Association of Surgeons of Great Britain and Ireland took place on 2–4 April 1986. The President of the Association, Mr Adrian Marston, was in the chair. This was a joint meeting with the Spanish Association of Surgeons under the presidency of Professor Benjamin Narbona.

The Moynihan Prize was awarded to J. A. Pain and M. E. Bailey for their paper 'Experimental and clinical study of lactulose in obstructive jaundice'.

Experimental and clinical study of lactulose in obstructive jaundice

J. A. Pain and M. E. Bailey

Department of Surgery, Royal Surrey County Hospital, Guildford, Surrey, UK

Correspondence to: Mr J. A. Pain

The role of lactulose in preventing endotoxaemia in obstructive jaundice has been investigated. A prospective study was performed on 24 consecutive patients with obstructive jaundice undergoing surgery. Twelve patients were given oral lactulose before operation and were compared with twelve controls. Endotoxaemia was reduced in peroperative portal ($P < 0.05$) *and postoperative systemic* ($P < 0.05$) *blood samples in the lactulose treated group, and a significant fall* ($P < 0.05$) *occurred in the postoperative 24 h creatinine clearances in controls compared with the lactulose treated group. Results from animal experiments in which oral lactulose reduced endotoxin related mortality in obstructive jaundice* ($P < 0.05$), *and the* in vitro *demonstration of a direct anti-endotoxic action of lactulose suggest that its beneficial action is due in part to an inactivation of endotoxin.*

Keywords: Disaccharides, endotoxin, limulus test, portal blood, bile duct obstruction – extrahepatic

Postoperative acute renal failure occurs in 9 per cent of patients with obstructive jaundice, and is associated with the presence of endotoxins in the systemic circulation[1]. Systemic endotoxaemia is believed to result from an increased absorption of intestinal endotoxin into the portal blood and a reduced clearance by the hepatic reticulo-endothelial system. The pre-operative oral administration of the bile salts, sodium deoxycholate and sodium taurocholate, have been demonstrated to prevent endotoxaemia and protect postoperative renal function in man[2,3], but the bile salts which are commercially available as gallstone disolution agents in the UK, ursodeoxycholic acid and chenodeoxycholic acid, have not been shown to be beneficial in human studies (Reference 4, J. A. Pain, unpublished data).

Lactulose, a non-toxic, synthetic disaccharide (galactoside-fructose), is frequently used in the treatment of constipation and prevention of hepatic encephalopathy. Several studies have shown that its oral administration prevents or abolishes systemic endotoxaemia[5–9], but its use has not been investigated previously in obstructive jaundice. The ability of lactulose to prevent endotoxaemia *in vitro* in animals with obstructive jaundice and in man has been studied.

Methods

In vitro *study*

Lactulose (Duphalac) was diluted with pyrogen-free water to concentrations ranging between 350 mg/ml and 0·35 mg/ml. One millilitre of the diluted lactulose was added to 0·8 ml sodium phosphate buffer. Twenty nanograms endotoxin (*Escherichia coli* 0111·B4, Difco) in 0·2 ml pyrogen-free water was added to the lactulose/buffer solution. After incubation at 37 °C for 6 h the detectable levels of endotoxin were measured with a quantitative limulus amoebocyte lysate (LAL) assay.

LAL assay. An endotoxin-free microwell strip (Dynatech Labs, USA) was placed on crushed ice and 0·1 ml of N181A LAL/N181B chromogenic substrate mixture (Mallinckrodt Inc, USA) was pipetted into each wall. Then 0·1 ml pyrogen-free water (standard blank) and 0·1 ml of endotoxin standards (100 pg, 50 pg, 25 pg, 12·5 pg, 6·25 pg/ml of *E. coli* 0111·B4, Difco, (USA) were used to construct a standard curve. The microwell strip was incubated on a heating block at 45 °C. After 35 min 0·05 ml of 50 per cent glacial acetic acid was added to each well simultaneously with a multichannel micropipette, and absorbances of the microwell strip were measured at 405 nm in a Titertek Multiskan spectrophotometer (Flow Labs, UK). Further 0·1 ml samples for endotoxin measurement were pipetted into wells containing LAL/chromogenic substrate and into wells containing 0·1 ml pyrogen-free water (sample blank), and then incubated and treated as above. Endotoxin concentrations of the samples were calculated from the standard curve.

Animal study

Forty-two male Wistar rats (250–300 g) underwent common bile duct ligation and division or sham operation, and were then allocated to one of five groups (*Figure 1*):

Group 1 ($n = 6$) Bile duct ligation for 14 days and then given oral endotoxin;
Group 2 ($n = 6$) Bile duct ligation for 14 days and then given intravenous lead acetate;
Group 3 ($n = 6$) Sham operation and 14 days later given oral endotoxin followed, 3 h later, by intravenous lead acetate;
Group 4 ($n = 12$) Bile duct ligation for 14 days and then given oral endotoxin followed, 3 h later, by lead acetate;
Group 5 ($n = 12$) Bile duct ligation for 11 days and then given oral lactulose for 3 days. On day 14 given oral endotoxin followed, 3 h later, by lead acetate.

Bile duct ligation and division or sham operation were performed via an upper midline incision under intraperitoneal pentobarbitone

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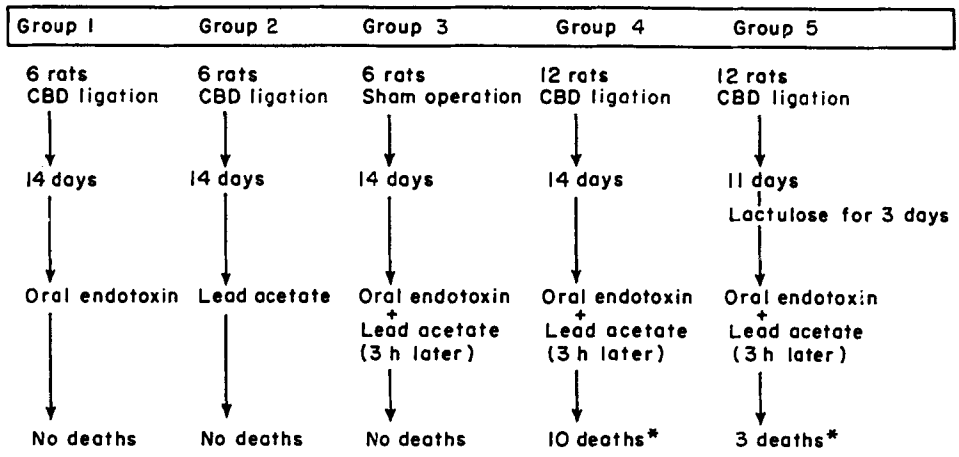

Figure 1 *Animal experiment. *Group 4–5: $\chi^2 = 6{\cdot}04$, d.f. = 1, $P < 0{\cdot}05$*

anaesthesia. The extrapancreatic part of the common bile duct was mobilized and divided between silk ligatures. In the sham operation a silk ligature was placed around the common bile duct and then removed. Throughout the experiment all rats were kept in a temperature-controlled environment and allowed water (except Group 5, while receiving lactulose) and food *ad libitum*.

Lactulose. Rats allocated to receive lactulose (Group 5) were given a 10 per cent solution of lactulose (Duphalac) to drink instead of water for three days (from days 12–14). In addition they were given 1 ml of undiluted lactulose twice daily via a gavage tube during these 3 days (Duphalac contains 67 g lactulose, 6 g lactose and 11 g galactose per 100 ml).

Oral endotoxin. Oral endotoxin, *E. coli* 0111·B4, was given via a gavage tube in a dose of 7·5 mg/100 g body weight.

Lead acetate. Lead acetate was injected into the rats under light ether anaesthesia into the dorsal vein of the penis in a dose of 7·5 mg/100 g body weight. Lead acetate causes a fatal toxicity in the presence of circulating endotoxins and was used as a bioassay to detect endotoxaemia. Mortality rates between the groups were assessed 30 h after the administration of lead acetate (or oral endotoxin in Group 1).

Patient study

A prospective study was performed on 24 consecutive patients with obstructive jaundice undergoing surgery. Twelve patients were given oral lactulose for 3 days before surgery and compared with twelve control patients.

Patients. All patients had a plasma bilirubin level > 100 μmol/l. The initial 12 patients entered into the study acted as controls and the subsequent 12 received lactulose. The diagnoses and operations performed are shown in *Table 1*. All patients received perioperative mannitol and antibiotics. There was no statistically significant difference (unpaired Student's *t* test) between the groups in any measured pre-operative parameter (sex, age, plasma bilirubin, plasma creatinine, alkaline phosphatase, aspartate amino transferase, albumin, haemoglobin, haematocrit, white cell count and platelets).

Lactulose. Oral lactulose (30 ml six hourly) was given for 3 days before surgery; the final dose being given within 9 h before surgery. If a patient developed troublesome or frequent diarrhoea (> 4 times per 24 h) the subsequent dose of lactulose was omitted and doses thereafter halved.

Endotoxin measurement. Blood was aspirated from a peripheral vein to measure systemic endotoxin levels one day before, during and on the first 3 days after surgery. Lithium-heparinized syringes (Monovette, Sarstedt) containing the blood samples were immediately placed on ice and, within one hour, platelet poor plasma was separated in a refrigerated centrifuge at 4 °C. In order to remove inhibiting and activating factors that interfere with the LAL assay the plasma was treated by diluting 1:9 with pyrogen-free water (Antigen, Ireland) and heated at 75°C for 5 min in pyrogen-free glass test tubes (Mallinckrodt Inc., USA). The treated plasma was then stored in the test tubes at −40 °C until analysis. Plasma endotoxin levels were measured using the LAL assay. As the plasma had been diluted 1:9 with water the endotoxin level calculated from the standard curve was multiplied by a factor of ten.

Table 1 *Diagnoses and operations performed*

	Control group ($n = 12$)	Lactulose group ($n = 12$)
Diagnosis		
carcinoma pancreas	7	8
carcinoma bile duct	2	1
Choledocholithiasis	3	3
Operation		
Palliative bypass	7	8
Whipple's operation	1	1
Stent insertion	1	0
Exploration common bile duct	3	3

Normal range and definition of endotoxaemia. Systemic blood samples were taken from 14 healthy patients admitted to hospital for routine minor surgery and 11 healthy laboratory staff. The mean (±s.e.m.) plasma endotoxin level in these controls was 41·6 + 6·8 pg/ml (range 0–112 pg/ml). Only one subject had a plasma endotoxin level above 100 pg/ml. For the purposes of this study endotoxaemia was defined as a plasma endotoxin level that was equal to or greater than 100 pg/ml (equivalent lipopolysaccharide *E. coli* 0111·B4).

Renal function. Serial 24 h endogenous creatinine clearance measurements (C24) were performed on the two days before surgery and for the first three postoperative days. Impairment of renal function was defined as either a pre-operative C24 of < 40 ml/min associated with raised plasma urea and creatinine levels or a > 20 per cent fall in mean C24 following operation. Renal failure was defined as a urine volume < 400 ml/24 h associated with a rising plasma urea and creatinine.

Statistical analysis

Paired and unpaired data were analysed using Student's *t* tests. Comparisons of the mortality rates in the animal study and incidences of endotoxaemia in the patient study were performed using the χ^2 test with Yates' correction.

Results

In vitro study

The minimum concentration of lactulose required to reduce the detectable endotoxin level from 20 ng/ml to less than 50 pg/ml (i.e. a 400-fold decrease) was 16·75 mg/ml.

Animal study

Jaundice was confirmed in rats by the production of dark urine and at post mortem examination. Two jaundiced rats from each group had bilirubin measurements performed in the second week after common bile duct ligation and the plasma levels were found to be > 150 μmol/l.

The rats receiving lactulose (Group 5) drank a mean of 29·4 ml/day (range 17–35 ml) of 10 per cent lactulose. Including the amount given by gavage tube the mean daily intake of

lactulose was 1·9 ml/100 g body weight. All rats developed soft or loose motions by the third day of lactulose treatment.

Jaundiced rats receiving either endotoxin (Group 1) or lead acetate (Group 2) all survived (*Figure 1*). Therefore given alone neither lead acetate nor oral endotoxin were lethally toxic to the jaundiced rat. Sham operated rats receiving both endotoxin and lead acetate (Group 3) all survived, whereas the jaundiced rats who received both substances (Group 4) had a mortality of 10/12. Lead acetate exerts its lethal toxicity in the presence of endotoxins, so it must only have been in the jaundiced rats that endotoxin was absorbed.

Only three of the twelve rats receiving lactulose before endotoxin and lead acetate died (Group 5). There was a statistically significant ($\chi^2 = 6{\cdot}04$, d.f. = 1, $P < 0{\cdot}05$) lower mortality in this group compared with Group 4 indicating that lactulose exerts a protective action by preventing endotoxaemia in rats with obstructive jaundice.

Patient study

Patients receiving lactulose had a mean 2·8 bowel actions per day, compared with 0·75 bowel actions per day in the control group. Owing to the frequency of the bowel actions doses of lactulose were omitted in five patients who subsequently took only 15 ml every 6 h.

There were three postoperative deaths in the control group due to pulmonary embolus, myocardial infarction and renal failure. In the lactulose group one patient died from intra-abdominal sepsis.

One patient in the control group, who had pre-operative renal impairment (C24 = 19 ml/min), developed postoperative renal failure and died on the seventh postoperative day. In the control group three patients had pre-operative and six patients had postoperative renal impairment. In the lactulose group two patients had pre-operative, but only one patient had postoperative renal impairment.

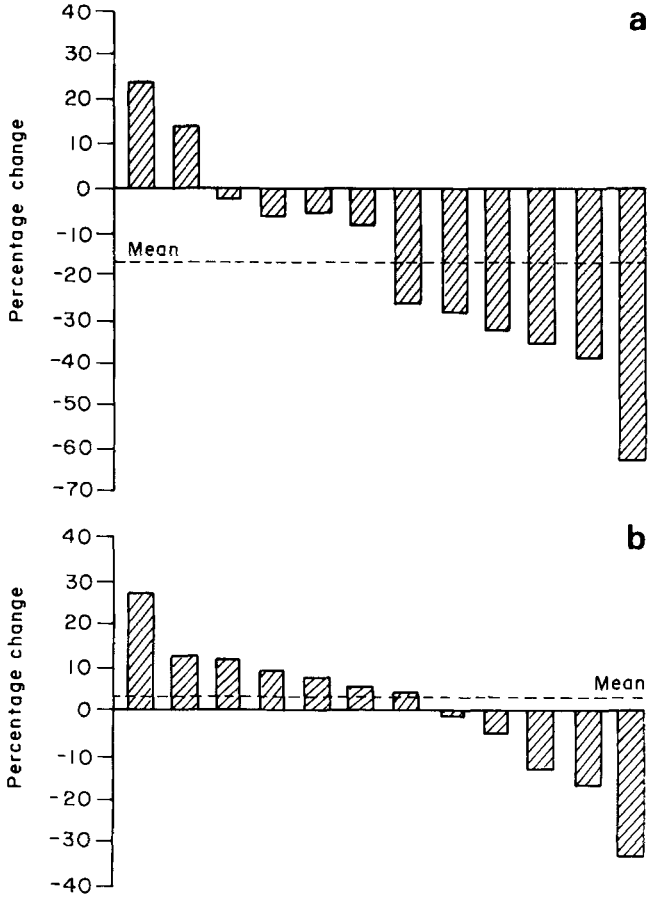

Figure 2 *Mean percentage change in creatinine clearance following operation for each patient.* **a** *Control group;* **b** *lactulose group*

Table 2 *Incidence of endotoxaemia*

	Control group ($n = 12$)	Lactulose group ($n = 12$)	Difference
Before operation	2	0	n.s.
At operation			
Portal	8	2	$\chi^2 = 4{\cdot}29$, d.f. = 1, $P < 0{\cdot}05$
Systemic	5	2	n.s.
After operation (× 3 days)	7	1	$\chi^2 = 4{\cdot}69$, d.f. = 1, $P < 0{\cdot}05$

*χ^2 with Yates' correction

Alterations in mean postoperative C24 compared with the pre-operative C24 are displayed in *Figure 2*. In the control group the overall mean pre-operative C24 was 63 ml/min and this fell in the first three postoperative days to 52 ml/min (17 per cent reduction). This was a statistically significant fall ($t = 2{\cdot}43$, d.f. = 11, $P < 0{\cdot}05$). In the lactulose group there was a 3 per cent rise from 67 ml/min to 69 ml/min following operation. In order to compare postoperative changes in C24 between the two groups a comparison was made of the mean alteration in C24 after operation for each patient. There was a statistically significant fall in the C24 in the control group compared with the lactulose treated group ($t = 2{\cdot}41$, d.f. = 20, $P < 0{\cdot}05$).

There were significantly fewer episodes of endotoxaemia (plasma endotoxin level > 100 pg/ml) in operative portal and postoperative systemic blood samples in the lactulose group compared with the control group (*Table 2*). Of the 36 systemic plasma samples taken after operation from each group 12 (33 per cent) from the control group had endotoxaemia compared with only two (6 per cent) from the lactulose treated group.

Discussion

Oral lactulose remains virtually unabsorbed. It exerts an osmotic load within the small intestine, and on reaching the colon it undergoes bacterial catabolism to form short chain carboxylic acids, so lowering the pH of the bowel contents. The resulting acidity increases colonic motility and this, combined with the increased osmotic load, constitutes the principal laxative actions of lactulose.

Reports from Italy[5,6] first suggested that lactulose could have a role to play in the treatment of endotoxaemia. Scevola *et al.*[6] reported that endotoxaemia was abolished in 95 per cent of patients with cirrhosis or active/chronic hepatitis after seven days treatment with oral lactulose and paromomycin (a non-absorbable antibiotic). A further study[5] compared lactulose alone, paromomycin alone and lactulose plus paromomycin in reducing endotoxaemia in viral hepatitis. The systemic blood endotoxin level was reduced in all three treatment groups but was lowest in the 'lactulose alone' group. Paromomycin may hinder the beneficial effects of lactulose because of the antibiotic's bacteriocidal action raising the free endotoxin level within the gut lumen[10], some of which could be absorbed into the portal vein blood. Oral lactulose has also been demonstrated to abolish systemic endotoxaemia in 50 per cent of patients with liver cirrhosis or hepatoma[7].

In animal experiments a single dose of oral lactulose prevents endotoxaemia resulting from ischaemic hepatic necrosis[9], and Liehr *et al.*[8] showed that oral lactulose prevents endotoxin induced galactosamine hepatitis in rats, however the experimental model used in this latter study has been criticized[11].

In the present study doses of lactulose similar to those recommended as an initial treatment of hepatic encephalopathy were given to patients. The only side effect was diarrhoea, and this necessitated a reduction in the dose of lactulose to five patients. Pre-operative oral lactulose reduced the incidence of

endotoxaemia in the operative portal and postoperative systemic blood, and prevented a fall in postoperative renal function in patients with obstructive jaundice. There was a statistically significant fall in the mean C24 following surgery in the control group compared with the lactulose treated group. Six patients in the control group had postoperative renal impairment and one of these patients died from renal failure. In the lactulose group only one patient developed postoperative renal impairment.

Lactulose may reduce endotoxin absorption either by a reduction or alteration of the gut flora thereby reducing the endotoxin pool available for absorption, by a direct effect on the endotoxin or by both methods. It has been suggested that bowel preparation to reduce the number of endotoxin releasing Gram-negative organisms and the gut endotoxin pool could decrease endotoxin absorption[12,13]. Although bowel preparation has not been found to be beneficial in obstructive jaundice[14], whole bowel irrigation does prevent endotoxaemia in patients with inflammatory bowel disease[15]. *In vitro* and animal experiments were performed to investigate the protective action of lactulose.

In the *in vitro* experiment lactulose produced a 400-fold decrease in the measurable level of endotoxin confirming previous findings that lactulose has a direct anti-endotoxic action[8,16]. Pre-treatment with oral lactulose reduced mortality in rats given both oral endotoxin and intravenous lead acetate. Selye *et al.*[17] observed that intravenous administration of non-lethal amounts of lead acetate to rats resulted in a 100 000-fold increase in their susceptibility to concurrently administered endotoxin, and is an accepted bioassay for detecting circulating endotoxins. Only minute amounts of lactulose are normally absorbed from the gastrointestinal tract so it is unlikely that it acts by altering the interaction of lead acetate and endotoxin. Endotoxin was given as a bolus just 3 h before lead acetate administration, and since lactulose prevented endotoxin absorption this would suggest that a substantial part of the beneficial action resulted from a direct effect on the endotoxin.

In addition to the *in vitro* and animal experiment findings the results from the prospective patient study suggest that lactulose may prove to be an effective agent in reducing renal failure and other endotoxin related complications in patients with obstructive jaundice. A prospective multicentre randomized study comparing pre-operative lactulose with bile salts in patients with obstructive jaundice is in progress.

Acknowledgements

The study was supported by Duphar Laboratories Ltd, the Mason Medical Research Foundation, the Smithkline Foundation and the Guildford Undetected Tumour Survey (GUTS). The authors are grateful to the surgeons of the Royal Surrey County Hospital, Guildford, and Heath Road Hospital, Ipswich, who allowed their patients to be studied.

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