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Lactulose as an antiendotoxin in experimental colitis

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The efficacy of lactulose as an antiendotoxin was studied and the effect of lactulose or colistin on faecal flora was investigated in a hapten-induced rat model of colitis. Enteral administration of lactulose to rats with colitis was associated with a significant reduction in the systemic concentration of endotoxin (median (range) 5·4 (0–19·9) *versus* 23·7 (0–145·0) pg/ml in colitic rats treated with water; 4·6 (0–10·8) pg/ml in healthy animals). Enteral administration of colistin significantly reduced the faecal count of aerobic Gram-negative bacilli (median (range) 2·84 (1·40–8·43) *versus* 8·26 (4·50–10·40) $\log_{10}$ colony-forming units per g faeces after treatment with water) but not the faecal load of endotoxin. Patients with inflammatory bowel disease may benefit from enteral treatment with lactulose to prevent systemic endotoxaemia and/or with colistin to modify enteric bacteria.

Luminal antigens, such as enteric bacteria and their products, may contribute to the pathogenesis of inflammatory bowel disease[1]. This hypothesis has been supported by reports of qualitative and quantitative changes in enteric bacterial flora[2,3], bacterial translocation[4,5], and raised concentrations of circulating antimicrobial antibodies[6] in patients with inflammatory bowel disease. Transmural migration of enteric bacteria may explain the pathogenesis of abscess and fistula in Crohn's disease and the high incidence of sepsis during elective surgery for inflammatory bowel disease[4], and may contribute to the biochemical and histological liver disturbances seen in these diseases[5]. Systemic endotoxaemia occurs in patients with active inflammatory bowel disease and correlates with the extent and activity of the disease[7–10]. Significantly raised titres of antibodies to the core region of bacterial endotoxin[10], to lipid A[11] and to peptidoglycan–polysaccharide complexes[12] have also been reported in patients with active inflammatory bowel disease.

Lactulose, which promotes the growth of lactobacilli, has been used successfully to treat intestinal infections[13] and systemic endotoxaemia[14,15]. Several possible explanations for the antiendotoxin action of lactulose have been suggested: (1) direct antiendotoxin activity[14,16,17]; (2) quantitative alteration in the faecal flora[18]; and (3) qualitative alteration in bacterial pathogenicity with reduced endotoxin production[16].

This study evaluated the efficacy of lactulose as an antiendotoxin in a hapten-induced model of colitis and investigated the effect of enteral lactulose or colistin (polymyxin E) therapy on the faecal bacterial and endotoxin load in this model.

Materials and methods

Animals

Male Wistar rats weighing 300–400 g were used. A standard rat pelleted formula and tap water were provided *ad libitum*.

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Induction of colitis

Colitis was induced in fasted and sedated animals by intracolonic instillation of 25 mg 2,4,6-trinitrobenzenesulphonic acid (TNBS) mixed with 0·25 ml 30 per cent ethanol as described previously[19]. A group of healthy animals ($n = 8$) and a group that received 0·5 ml 0·9 per cent saline by intracolonic instillation ($n = 8$) were also studied.

Study 1: lactulose and systemic endotoxaemia

Treatment regimen. Commencing 5 days after induction of colitis and continued twice daily for 3 days the rats received by gavage either 2 ml undiluted Duphalac (67 g lactulose, 6 g lactose and 11 g galactose per 100 ml; Duphar Laboratories, Southampton, UK) ($n = 16$) or an equal volume of tap water ($n = 16$). For rats receiving lactulose drinking water was replaced by a lactulose solution (100 ml Duphalac per litre water)[14].

Systemic endotoxin concentration. Plasma was obtained on day 8 and assayed for endotoxin using a quantitative chromogenic *Limulus* amoebocyte lysate assay (Coatest Endotoxin; Kabi Diagnostica, Molndal, Sweden)[19]. The assay has a sensitivity of 8·3 pg/ml and is linear from 8·3 to 100 pg/ml.

Assessment of colitis. On day 8, severity of inflammation was assessed by colon weight, colon macroscopic scoring and histological examination as described previously[19].

Study 2: faecal flora and endotoxin load

Treatment regimen. On day 5 after induction of colitis, rats were randomly allocated to receive lactulose ($n = 8$), colistin ($n = 8$) or water ($n = 8$). The drinking water was replaced by a solution of lactulose (100 ml Duphalac per litre water), colistin sulphate (1 g Colomycin (Pharmax, Bexley, UK) per litre water) or water (control group). Rats in the lactulose group received 1 ml undiluted Duphalac by gavage. Therapy was continued for 72 h.

Systemic blood culture. At 8 days after induction of colitis, systemic blood was taken for aerobic and anaerobic culture using the radiometric Bactec 460 method (Becton Dickinson, Towson, Maryland, USA). Blood cultures were read on days 1, 2, 4 and 7 at a threshold of 20 and 30 for anaerobes and aerobes respectively. Positive blood cultures were plated out on appropriate media and identified by standard bacteriological techniques.

Collection of faecal samples. Fresh faecal samples were collected for quantitative bacterial culture[20] and determination

of endotoxin concentration using the *Limulus* assay. Severity of colitis was assessed as above.

Statistics

Data analysis was performed on a Macintosh LC computer (Apple, Cupertino, California, USA) using χ^2, Student's *t* and Mann–Whitney *U* tests (Statworks; Cricket Software, Philadelphia, Pennsylvania, USA). Probabilities of less than 0·05 were considered to be significant.

Results

Clinical features

The rats with colitis showed weight loss, diarrhoea, piloerection, reduced fluid intake, lack of preening, and a reduced level of activity. There were no deaths.

Study 1: lactulose and systemic endotoxaemia

Colonic inflammation. Significant inflammation of the colon was induced in the rats with colitis compared with saline-treated animals or healthy controls (*Table 1*). Histological features of this colitis have been described previously[19,20]. No qualitative differences were observed in the histological appearance of colon from the different treatment groups.

Systemic endotoxaemia. Enteral lactulose therapy produced a significant reduction in systemic endotoxaemia compared with that seen in rats given water (*Table 1*).

Study 2: faecal flora and endotoxin load

Assessment of colitis. There was no significant difference in the severity of the colitis between the different treatment groups (*Table 2*).

Systemic blood cultures. Positive systemic blood cultures were obtained in seven animals. There was no significant difference between treatment groups (*Table 2*). The commonest organism cultured was *Escherichia coli*, which was present in four positive cultures.

Aerobic and anaerobic faecal flora. For rats allocated to receive water induction of colitis was associated with no significant change in the faecal concentration of aerobic or anaerobic bacteria compared with healthy controls (*Figs 1* and *2*). In comparison to those given water, colistin therapy for 72 h produced a significant reduction in the faecal count of aerobic Gram-negative bacilli (*Fig. 1*).

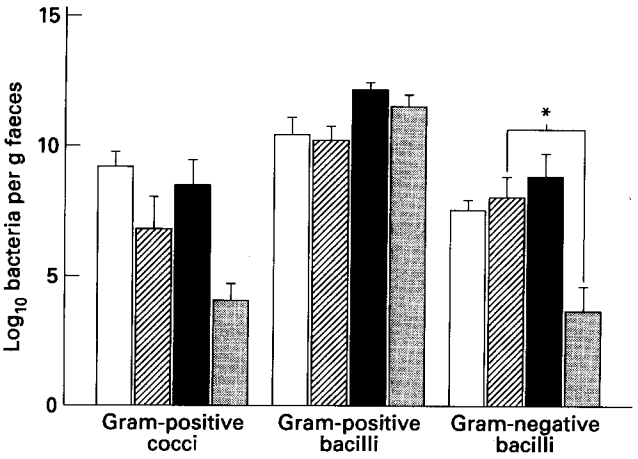

Fig. 1 Median (range) faecal concentration of aerobic bacteria before treatment (□) and in rats treated with water (▨), lactulose (■) or colistin (▩) for 72 h. *$P < 0·05$ (Mann–Whitney *U* test)

Table 1 Study 1: colonic inflammation assessment and systemic endotoxin concentration

Treatment group	*n*	Indices of colonic inflammation*			Systemic endotoxin (pg/ml)†
		Colon macroscopic score	Colon weight (g)	Colon weight (g)/ body-weight (kg)	
Rats with colitis					
Lactulose	16	4·59(0·51)‡	1·54(0·11)**	4·33(0·27)**	5·4 (0–19·9)§
Water	16	4·17(0·61)‡	1·63(0·39)**	4·98(1·30)**	23·7 (0–145·0)‡
Intracolonic saline	8	0·31(0·13)	0·74(0·03)	1·88(0·05)	2·1 (0–13·7)
Healthy controls	8	0·38(2·0)	0·72(0·10)	1·58(0·23)	4·6 (0–10·8)

Values are *mean(s.e.m.) or †median (range). ‡$P < 0·05$ *versus* intracolonic saline group (Mann–Whitney *U* test); **$P < 0·05$ *versus* intracolonic saline group (Student's *t* test); §$P < 0·05$ *versus* rats with colitis treated with water (Mann–Whitney *U* test)

Table 2 Study 2: colitis assessment, faecal endotoxin load and blood culture results

Treatment group	*n*	Indices of colonic inflammation*			Endotoxin load ($\times 10^6$ μg per g faeces)†	Positive blood culture
		Colon macroscopic score	Colon weight (g)	Colon weight (g)/ body-weight (kg)		
Rats with colitis						
Water	8	4·0(1·0)	2·2(0·8)	7·1(2·6)	10·4 (4·5–40·6)	3
Lactulose	8	4·7(0·9)	1·9(0·4)	5·4(1·2)	20·3 (17·2–50·7)	3
Colistin	8	3·7(0·7)	1·1(0·2)	3·7(0·5)	12·5 (5·0–22·9)	1
Healthy controls	8	0·4(0·2)	0·7(0·1)	1·6(0·2)	31·5 (15·7–44·8)	0

Values are *mean(s.e.m.) or †median (range). There was no significant difference between treatment groups for each parameter

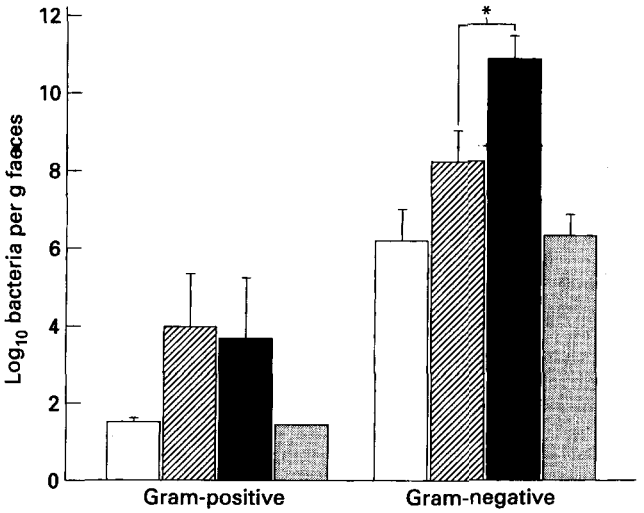

Fig. 2 Median (range) faecal concentration of anaerobic bacteria before treatment (□) and in rats treated with water (▨), lactulose (■) or colistin (▩) for 72 h. *$P < 0.05$ (Mann–Whitney U test)

There was a significantly higher faecal anaerobic Gram-negative count in rats receiving lactulose therapy compared with the controls given water (*Fig. 2*).

Colonic endotoxin load. There was no significant difference in faecal endotoxin concentration between healthy animals and rats with colitis in any of the treatment groups (*Table 2*).

Discussion

Endotoxaemia of intestinal origin is thought to occur when there is an alteration in endotoxin production, absorption and/or detoxification[21]. Clinical studies have revealed that if the gut mucosal barrier is damaged by inflammatory bowel disease, colonoscopy or colonic neoplasia systemic endotoxaemia results[22]. Systemic endotoxaemia has also been shown to occur in spontaneous and induced enterocolitis in experimental animals and to correlate with the severity of the colitis and systemic illness[23,24]. In the present studies, colitis was induced in rats by the intracolonic instillation of ethanol to break down the colonic mucosal barrier in combination with the hapten TNBS[25]. This model of transmural chronic colitis has been investigated extensively and found to have clinical, biochemical and histological similarities to colonic Crohn's disease[19,20,25].

In the first part of the study, administration of lactulose to animals with colitis prevented systemic endotoxaemia. Lactulose has also been shown to reduce systemic endotoxaemia in clinical and experimental obstructive jaundice, viral hepatitis, liver cirrhosis and experimental ischaemic hepatic necrosis[14,15].

TNBS-induced colitis is also associated with reduced faecal Gram-positive cocci and increased aerobic Gram-negative bacilli counts[20]. Indigenous enteric anaerobic Gram-positive bacilli are thought to be important for the body to resist invasion by potentially pathogenic Gram-negative bacilli[26]. Two therapeutic strategies might be useful in preventing the transmural migration of bacteria and bacterial endotoxin in experimental colitis: the promotion of faecal Gram-positive anaerobic bacilli such as *Lactobacillus* species by lactulose or the selective elimination of aerobic Gram-negative bacilli using colistin. In the second study, administration of lactulose to animals with colitis failed to cause any increase in faecal Gram-positive anaerobic bacilli or any significant reduction in faecal endotoxin load or bacteraemia. In support of this, Bircher *et al.*[27] have found that lactulose is slow to cause changes in the faecal flora.

Enteral colistin therapy significantly reduced the count of faecal Gram-negative bacilli in rats with colitis, without reducing faecal endotoxin load or bacteraemia. Colistin therefore fulfills the criteria of van der Waaij[28] for selective digestive decontamination, in being able to suppress potentially pathogenic Gram-negative bacilli without significantly reducing the anaerobic flora. This is in agreement with Goris *et al.*[29] who found that treatment for 1 day with polymyxin E (1 g per litre water) completely suppressed faecal aerobic Gram-negative bacteria in C_3H/Law mice. Van der Waaij *et al.*[30] also showed that selective elimination of aerobic Gram-negative bacilli, using trimethoprim and sulphamethoxazole, significantly reduced the ulcerative lesions developing in guinea pigs treated with 2 per cent degraded carrageenan. In a similar study, Onderdonk *et al.*[31] found that pretreatment with metronidazole prevented carrageenan-induced colitis in the majority of guinea pigs studied but that treatment with metronidazole starting after the onset of colitis was of no benefit.

Induction of colitis by intracolonic instillation of TNBS and ethanol is associated with a disturbed colonic flora and systemic endotoxaemia[19,20]. Enteral administration of colistin reduced the concentration of faecal Gram-negative bacilli in TNBS-induced colitis. Lactulose therapy, however, while preventing systemic endotoxaemia in this model, did not appear to alter faecal aerobic Gram-negative bacilli either quantitatively or qualitatively. A direct effect of lactulose on endotoxin in the colon seems unlikely because of its metabolic conversion by colonic bacteria to short-chain fatty acids. A further possibility is that there is increased absorption of lactulose in TNBS–ethanol-induced colitis because of a generalized increase in colonic permeability[32] and that lactulose is exerting a systemic antiendotoxin action. A natural extension to these studies and to those involving patients with liver disease[14,15] would be to evaluate the use of lactulose in patients with inflammatory bowel disease either as an antiendotoxin during active disease or as prophylaxis against relapse induced by bacterial products. Although lactulose is generally tolerated well[14,15], it may prove less acceptable to patients with inflammatory bowel disease who are suffering from severe diarrhoea than to those with obstructive jaundice, hepatitis or cirrhosis.

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