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ORIGINAL ARTICLE

The probiotic bacterium *Lactobacillus plantarum* species 299 reduces intestinal permeability in experimental biliary obstruction

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Abstract

Aims: Extrahepatic biliary obstruction is associated with the failure of intestinal barrier function, allowing bacteria and other substances from the intestine to enter the circulation and initiate a systemic inflammatory response, causing impairment of organ function. Probiotic bacteria have been shown to have beneficial effects on intestinal barrier function in other conditions, but their effects have never been studied in biliary obstruction.

Methods and Results: This study examined the effects of enteral administration of *Lactobacillus plantarum* species 299 (LP299) in oatmeal fibre compared with sterile oatmeal fibre in water or water alone in an animal model of biliary obstruction. Administration of LP299 was associated with reduced intestinal permeability compared with sterile oatmeal alone ($0{\cdot}262 \pm 0{\cdot}105\%$ *vs* $0{\cdot}537 \pm 0{\cdot}037\%$, $P = 0{\cdot}019$, percentage excretion of ^{14}Carbon), but there was no evidence of reduced endotoxin exposure or blunting of the systemic inflammatory response. Animals receiving sterile oatmeal fibre alone also failed to develop the hyperpermeability after biliary obstruction seen in animals receiving water only ($0{\cdot}512 \pm 0{\cdot}05\%$ *vs* $0{\cdot}788 \pm 0{\cdot}18\%$), suggesting that oatmeal itself may have some beneficial effects on intestinal barrier function.

Conclusion: Enteral administration of the probiotic bacterium LP299 reduces intestinal hyperpermeability associated with experimental biliary obstruction.

Significance and Impact of the Study: This study provides insight to direct further work into the modulation of intestinal barrier function by probiotic bacteria.

Introduction

Recent research has suggested that extrahepatic biliary obstruction in humans and animals is associated with disruption of intestinal integrity (Parks *et al.* 2000) and increased intestinal permeability (IP) (Parks *et al.* 1996a,b, 2003; Welsh *et al.* 1998) The resulting systemic endotoxaemia is thought to stimulate a systemic inflammatory response syndrome (Clements *et al.* 1998), leading to the dysfunction of multiple organ systems which is responsible for the complications associated with biliary obstruction. This sequence of events might be prevented by therapies aimed at improving intestinal barrier function.

The human gastrointestinal tract contains approx. 10^{12} bacteria in total, comprising over 400 species. Less than 1% of the total number are potentially harmful, endotoxin-containing Gram-negative aerobes such as *Escherichia coli* (Swank and Deitch 1996). The normal composition of the intestinal microflora is known to be altered in experimental biliary obstruction: a number of authors have demonstrated overgrowth of Gram-negative species in the distal ileum and colon after bile duct ligation (BDL) (Clements *et al.* 1996; Parks *et al.* 1996a,b;

Nieuwenhuijs *et al.* 2000). Changes in luminal microbial balance may contribute to intestinal barrier failure and administration of Gram-negative species has been shown to increase IP under experimental conditions (Garcia-Lafuente *et al.* 2001).

Probiotic bacteria are defined as live, orally administered micro-organisms which exert a beneficial effect on the host by altering the composition of the luminal microflora (Fuller 1989; Saavedra 2001). These bacteria are normally present in the gastrointestinal tract and have a digestive function in breaking down fibre and complex macromolecules by fermentation (Bengmark 1998; Rolfe 2000). The probiotic bacterium *Lactobacillus plantarum* species 299 (LP299) has been shown to improve intestinal barrier function in a range of experimental models of colitis, pancreatitis and liver injury, but no studies have examined its effects in biliary obstruction (Fabia *et al.* 1993; Wang *et al.* 1995; Kasravi *et al.* 1997; Mangiante *et al.* 1999; Schultz and Sartor 2000; Mangiante *et al.* 2001).

In summary, the literature to date suggests that the composition of the luminal flora is an important determinant of IP and that altering the intestinal flora using a probiotic bacterium such as *L. plantarum* may improve intestinal barrier function in biliary obstruction. The aim of this study was to test the hypothesis that enteral administration of LP299 would improve intestinal barrier function in an experimental animal model of biliary obstruction.

Materials and methods

Oatmeal fibre containing live LP299 was obtained from AB Probi (Lund, Sweden). Solutions were prepared containing 0·4 g fibre and 10^9 live LP299 organisms per millilitre, confirmed using serial dilution and culture and optical densitometry measurements. Solutions containing an identical amount of sterile oatmeal fibre were used for comparison. Thirty 9-week-old male Wistar rats (approx. 300 g body weight) were randomized into three groups to receive either oatmeal fibre + LP299 ($n = 10$), sterile oatmeal fibre alone ($n = 10$) or tap water ($n = 10$). These solutions were administered daily for 16 days using an orogastric gavage tube with each animal receiving 3·5 ml of the appropriate solution per day. All animals were given access to SDS-RM3A rat food (Special Dietary Services, Witham, UK) and tap water; animals in the LP299 group were pair-fed with animals in the oatmeal fibre and water groups. Experiments were performed over a 16-day period, with all animals undergoing BDL on day 9 as described below. IP was estimated on the day before BDL (day eight) and again 7 days after BDL (day 15) by estimation of the 24-h urinary excretion of 1·1 μCi of orally administered ^{14}carbon-labelled PEG as described by Ryan *et al.* (1993). Urinary ^{14}carbon activity was determined using a scintillation counter and IP was expressed as percentage excretion of the orally administered dose of ^{14}carbon. Initial studies had determined that ^{14}carbon activity in the urine decreased to background levels within 96 h of oral administration, and did not accumulate in the urine after repeated doses.

Bile duct ligation and division (BDL) was carried out under an anaesthetic of ketamine (10 mg/100 g body weight, intramuscular) and xylazine (1 mg/100 g, subcutaneous). The abdomen was opened and the bile duct divided below the confluence of the lobar hepatic ducts between 4/0 silk ligatures. The abdomen was closed in two layers using a 3/0 chromic catgut suture. Buprenorphine (3 μg/100 g) was administered intramuscularly for postoperative pain control and animals were allowed food and water postoperatively under the pair-feeding conditions described above. After the final IP estimation, a terminal anaesthetic was administered and samples of serum and terminal ileum were collected. The serum was analysed to determine parameters of hepatic function and also for bioactive interleukin-6, as a marker of the systemic inflammatory response, using IL-6-dependent B9 hybridoma cells (Wortel *et al.* 1993). Endogenous antibody to the core region of endotoxin was also estimated as an indicator of endotoxin exposure, using an enzyme-linked immunosorbent assay measuring antibody to the core gycolipid region of LPS (Barclay 1995). Terminal ileal morphology was assessed using computerized morphometric analysis. All experiments were carried out in accordance with the United Kingdom Animals Scientific Procedures Act of 1986.

Statistical comparisons were made between the LP299, oatmeal fibre and water groups. Body weight changes and mucosal morphometric measurements were compared using the Student's *t*-test for independent variables. IP measurements were compared by analysis of variance for repeated measures, with *post hoc* comparison of groups using the *t*-test for independent variables with results expressed as mean ± SE. The data for antiendotoxin antibody levels and IL-6 estimation were not normally distributed and thus were analysed using the Kruskal–Wallis procedure and the Mann–Whitney *U*-test, with results expressed as medians (interquartile range). Statistical significance was accepted at the 5% level.

Results

Of the 30 animals, one failed to recover from BDL and two others were killed after failing to eat and drink. There were no significant differences in food or water consumption or in body weight at any time point between groups.

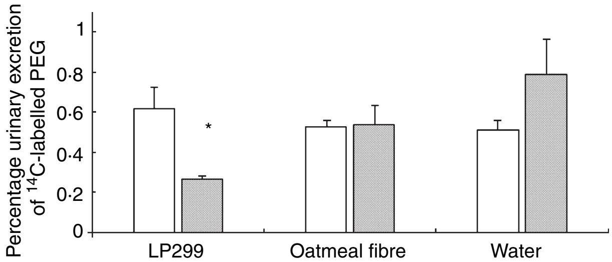

Figure 1 Intestinal permeability changes in LP299, oatmeal fibre and water groups [light bars: before bile duct ligation (BDL); dark bars: 7 days after BDL; all results are mean ± SEM. **P* = 0·019 *vs* oatmeal fibre].

The mean serum bilirubin in all animals was significantly elevated after BDL (105 ± 4 μmol l^{-1}), confirming biliary obstruction.

Repeated measures ANOVA showed that there were significant differences in ^{14}carbon excretion between the groups 1 week after BDL ($P < 0·05$), indicating an increase in IP associated with biliary obstruction. BDL was noted to be associated with a rise in IP in animals receiving water alone. *Post hoc* analysis indicated that percentage excretion of ^{14}carbon PEG 1 week after BDL was significantly lower in animals receiving LP299 in oatmeal, than in those receiving oatmeal fibre alone (Fig. 1 and Table 1).

There were no differences detected between the LP299 and oatmeal fibre groups in mucosal morphometry, anti-endotoxin antibody levels (Table 1) or in serum IL-6 levels (median value in both groups 0 pg ml^{-1}, detected in one of eight in LP299 group and two of nine in sterile oatmeal fibre group). Similarly, there were no differences in these variables between either of these groups and the water group (Table 1).

In summary, enteral administration of LP299 in oatmeal was associated with reduced IP after BDL when compared with administration of oatmeal alone. LP299 administration was not associated with any alterations in serum IL-6 levels, mucosal morphometry or exposure to endotoxin.

Discussion

The possible health benefits of probiotic bacteria were first investigated by Metchnikoff nearly 100 years ago, in a study of the link between longevity and the consumption of fermented milk in Bulgarian peasants (Gibson and Fuller 1998). *Lactobacillus* was isolated from this milk and is one of the four strains now most commonly considered as probiotic bacteria: the Gram-positive *Lactobacillus*, *Lactococcus*, *Streptococcus* and *Bifidobacterium*.

This study is the first to demonstrate that enteral administration of the probiotic bacterium LP299 prevents the development of intestinal hyperpermeability associated with experimental biliary obstruction. This finding is in agreement with two previous studies which demonstrated that administration of *LP299* inhibits *E. coli*-induced intestinal hyperpermeability in rat intestine (Garcia-Lafuente *et al.* 2001; Mangell *et al.* 2002). The exact mechanism by which *Lactobacillus* strains produce this effect is unknown, but may be related to the production of trophic factors and nutrients such as short-chain fatty acids, polyamines, and nitric oxide which maintain enterocyte structure and influence mucosal immune function (Scheppach 1994; Duncan *et al.* 1995; Bengmark 2000). It may also be related to beneficial alterations in the intestinal microflora which LP299 may bring about by the secretion of antimicrobial substances which prevent the overgrowth of other species and prevent the attachment and translocation of pathogenic Gram-negative bacteria from the intestine (Silva *et al.* 1987; Coconnier *et al.* 1993, 1997; Isolauri *et al.* 1993; Bernet *et al.* 1994; Bernet-Camard *et al.* 1997; Fuller and Gibson 1997; Todoriki *et al.* 2001). There is also evidence that LP299 may exert an direct immunomodulatory effect on mucosal cells (Michail and Abernathy 2002, 2003; Pathmakanthan *et al.* 2004). Although this study did demonstrate a reduction in IP in animals treated with LP299, there was no evidence of reduced endotoxin

Table 1 Summary of experimental results

	LP299	Oatmeal fibre	Water
Intestinal permeability (%)			
Before bile duct ligation (BDL)	0·618 ± 0·105	0·524 ± 0·037	0·512 ± 0·045
7 days after BDL	0·262 ± 0·019	0·537 ± 0·097	0·788 ± 0·177
Mucosal morphometry (mean ± SEM)			
Villus height : crypt depth ratio	1·483 ± 0·075	1·446 ± 0·053	1·920 ± 0·058
Total mucosal depth (mm)	0·436 ± 0·019	0·411 ± 0·015	0·361 ± 0·012
Endogenous antibody to endotoxin, median (interquartile range)			
IgG (% of standard)	398 (242–540)	329 (274–389)	302 (126–493)
IgM (% of standard)	356 (228–482)	257 (163–277)	183 (141–475)

exposure or of blunting of the systemic inflammatory response. This may have been due to the effects of short-chain fatty acids produced by intestinal breakdown of the oatmeal fibre given in the oatmeal fibre group. This may also account for the fact that BDL in the oatmeal fibre group was not associated with the expected 50% rise in IP which we have previously demonstrated (Parks *et al.* 1996a,b) and which was observed in animals receiving water alone in the present study. Short-chain fatty acids produced from oat fibre have been shown to improve intestinal structure and function (Scheppach 1994), and it may be that they are responsible for the effects observed in the oatmeal fibre group. The additional permeability changes observed after administration of LP299 in oatmeal compared with oatmeal fibre alone do however indicate that LP299 is capable of reducing IP to a greater extent than oatmeal fibre alone. Further work should focus on eludicating the mechanisms by which probiotic bacteria alter the gut micro-environment and influence intestinal barrier function.

In conclusion, this short study supports the hypothesis that the enteral administration of *L. plantarum* species 299 is associated with reduced IP in experimental biliary obstruction. Further work is required to eludicate the mechanisms by which probiotic bacteria alter the gut micro-environment and influence intestinal barrier function.

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