Lysophosphatidylcholine increases rat ileal permeability to macromolecules

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SUMMARY The influence of lysophosphatidylcholine (LPC) on macromolecular permeability in the distal ileum has been studied. Using a rat experimental model, we determined the intestinal permeability to different sized dextrans (3000–70 000 daltons) and bovine serum albumin (BSA) in the absence and presence of LPC. We also examined the morphology of the ileal mucosa after deposition of LPC in the gut lumen, and determined N-acetyl- β -glucosaminidase, 5'-nucleotidase, and alkaline phosphatase activities in suspensions of isolated mucosal cells and different concentrations of LPC. We found that 20 mM LPC damaged the ileal mucosa and that it increased its permeability to all the molecules investigated. Moreover, mixtures of mucosal cells and 0.01–1 mM LPC showed increased N-acetyl- β -glucosaminidase activity: the higher the LPC concentration, the higher the enzyme activity. These findings indicate that LPC, a naturally occurring surfactant in the intestine, might damage mucosal cells and release lysosomal enzyme activity, and that higher LPC concentrations may impair the mucosal barrier function and increase the gut permeability to macromolecules such as proteins. This could have relevance to the development of various disease states, in which increased intestinal absorption of macromolecules is of importance.

Intestinal absorption of macromolecules may be of critical importance to the pathogenesis of many diseases, especially those involving allergic reactions to ingested foreign substances or toxic reactions to bacterial and parasitic products.¹ Little is known, however, about the mechanisms by which the absorption of macromolecules could be facilitated in various disease states. Chadwick *et al*² recently showed that some of the naturally occurring bile acids in man can alter rabbit colonic structure and function, and that bile acid induced fluid secretion was invariable associated with epitheliolysis and increased mucosal permeability. Alternatively, we have found that another naturally occurring detergent, lysophosphatidylcholine (LPC), can impair the mucosal barrier in the distal part of rat ileum and thus enable larger molecules such as dextran 3000 to traverse the intestinal wall.³ The detailed mechanisms behind this effect are not known, however, and it is therefore unclear whether LPC may increase also the permeability to larger

macromolecules such as proteins.

The present study was conducted with a view to obtain more detailed information concerning how LPC might influence intestinal permeability to larger molecules. We therefore investigated the influence of LPC on rat intestinal permeability to larger dextrans (3000–70 000 daltons) and to bovine serum albumin, a 70 000 dalton protein. In addition, we examined the morphology of the ileal mucosa after deposition of LPC in the bowel lumen, and determined N-acetyl- α -glucosaminidase, 5'-nucleotidase, and alkaline phosphatase activities in mixtures of isolated mucosal cells and LPC. The results indicate that LPC may act as a detergent and release lysosomal enzymes from mucosal cells *in vitro*, and that higher LPC concentrations increase the shedding of enterocytes at the villous tips and augment the mucosal permeability to dextran 70 000 and bovine serum albumin.

Methods

CHEMICALS

Fluorescein-isothiocyanate (FITC) conjugated

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dextrans with molecular weights of 3000 and 10 000 daltons were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden, whereas FITC-dextran 70 000 was bought from Sigma Chemical Company, St Louis, Missouri, USA. Lyso-phosphatidylcholine (1-acyl-*sn*-glycero-3-phosphocholine) was Sigma type I, whereas bovine serum albumin was Sigma type V.

PERMEABILITY MEASUREMENTS

All experiments were carried out on 225–250 g female Sprague-Dawley rats maintained on a standard laboratory diet. They were anaesthetised by an intraperitoneal injection of Ketalar® (Parke, Davis, Pontypool, UK; 50 mg/kg body weight) and Rompun® (Bayer AG, Leverkusen, West Germany; 5 mg/kg body weight). A polyethylene catheter ($\emptyset = 0.96$ mm) was placed in one of the carotid arteries. Thereafter, a 10 cm segment of the distal ileum immediately proximal to the caecum was converted to a tied loop. Before ligating the proximal end, a flexible cannula with an injection valve was put into the segment through a separate insertion. The permeability markers (FITC-dextran 3000, 10 000, or a mixture of FITC-dextran 70 000 and bovine serum albumin) were dissolved in 1 ml 150 mM NaCl together with (or without) 20 mM LPC or 1% Triton X-100, and instilled into the loop. Blood samples (200 μ l) were withdrawn from the carotid artery before instillation and at intervals thereafter. The samples were analysed for FITC-dextran and/or bovine serum albumin.

To determine the concentration of FITC-dextran, the sample was mixed with 3.8 ml Tris (50 mM)-NaCl (150 mM), pH 10.3, and centrifuged at 2800 g for five minutes. The supernatant was then analysed for FITC-dextran by using fluorescence spectrometry as described elsewhere.³ Bovine serum albumin in serum was determined by electro-immunoassay using crystallised bovine serum albumin (Sigma type A 7638) as standard.⁴ Specific antiserum to bovine serum albumin was obtained from Miles Laboratories (Freehold, USA).

MORPHOLOGY

Tied loops prepared as above were either left unfilled or filled with 1 ml 150 mM phosphate buffered saline (PBS), 20 mM LPC in 1 ml PBS, or 1% Triton X-100 in 1 ml PBS. After 30 minutes, 300 ml 2% glutaraldehyde in 0.1 M Na-cacodylate-HCl with 0.1 M sucrose and 2% polyvinylpyrrolidone (PVP, molecular weight 44 000 daltons) was perfused *via* the heart.^{5,6} The perfusion was conducted during 10 minutes at a constant hydrostatic pressure of 110 mmHg. The pH of the fixative was 7.2, its temperature 37°C and its total

osmolality about 560 mOsm (vehicle osmolality about 300 mOsm).

For light microscopy, five 2 mm thick segments were sampled from each loop and immersed in the above fixative for another three days at 4°C. The specimens were then dehydrated, embedded in Sorwall JB4 resin, cut into 3 μ m thick sections and stained with either haematoxylin eosin, haematoxylin van Gieson, or 0.5% toluidine blue at pH 4.

The morphological changes in villous tips were quantitatively assessed by examining intestinal circumferences in sections stained with haematoxylin van Gieson. Tips were thus classified into three categories according to their morphological appearance: (i) normal morphology with regular columnar epithelium, (ii) moderately changed morphology with loss of columnar characteristics, partial desquamation, karyolysis and karyorrhexis, and (iii) severely changed morphology with patchy desquamation of epithelium leaving denuded areas of basal membrane. A mean of 36 villi (range 22–69) were examined in each circumferential section.

For transmission electron microscopy, two 1 mm wide segments of each loop were immersed in the above fixative for another three days at 4°C. Three 1 mm cubic pieces were taken from each segment, rinsed in 0.15 M Na-cacodylate-HCl buffer (CAC) and postfixed in 1% OsO₄ in 0.15 M CAC. After another rinse in 0.15 M CAC, the specimens were 'stained' in 2% uranyl acetate in 50% ethanol over night. After dehydration in a graded ethanol series and embedding in Epon 812, the specimens were cut with diamond knives on a LKB III ultratome, stained with lead citrate and examined in a Philips 300 electron microscope at 60 kV.

INFLUENCE ON MUCOSAL CELLS IN VITRO

The distal ileum (about 20 cm) was excised and the faecal contents removed. The mucosa was then scraped off with a curette. Special precaution was taken to scrape only the superficial layers of the mucosa. The cells were suspended in Krebs-Ringer phosphate buffer containing 10 mM glucose (KRG) and 0.5% (w/v) trypsin to a concentration of 15 mg tissue/ml. This suspension was subjected to magnetic stirring for 30 minutes, Vortex-mixed for 1 minute, and filtered through gauze. The filtrate was centrifuged (800 g, 10 min), the supernatant discarded, and the pellet resuspended in KRG. One hundred microlitres of this cell suspension was then mixed with 100 μ l LPC (final concentration 0.01–1 mM), and the mixture incubated at room temperature for 30 minutes. The activities of alkaline phosphatase,⁷ 5'-nucleotidase^{8,9} and N-

acetyl- β -glucosaminidase^{8 10} were then determined. To study the influence of LPC on non-lysosomal N-acetyl- β -glucosaminidase, the mucosal cells were disintegrated in a glass homogenizer, subjected to three freeze-thawing cycles, and centrifuged at 30 000 g for 30 minutes. The supernatant was then withdrawn and used for further experimentation. The material was incubated with varying concentration (0.001–10 mM) LPC as described above, and the activity of N-acetyl- β -glucosaminidase determined.

STATISTICAL DETERMINATIONS

Statistical analysis were carried out with the use of Student's *t* test.

Results

INFLUENCE OF LPC ON INTESTINAL PERMEABILITY

The intestinal absorption into blood of different sized dextrans in the absence and presence of LPC is illustrated in Figure 1. After deposition in the absence of LPC, only minute amounts of dextran 3000 were found in the circulation, whereas in the presence of LPC considerable amounts were found. Lysophosphatidylcholine also enhanced the transfer of dextran 10 000, although as shown in Figure 1, the transfer of dextran 10 000 was less than that of

dextran 3000. For comparison, the influence of Triton X-100, a non-ionic detergent, on the permeability to dextran 3000 and 10 000 is also shown in Figure 1. Thus, like LPC, Triton X-100 significantly augmented the permeability both to dextran 3000 and 10 000 (Fig. 1, right).

The influence of LPC on the transfer of dextran 70 000 and bovine serum albumin is illustrated in Figure 2. We thus found that the presence of LPC significantly increased the absorption also of these two compounds.

INFLUENCE ON INTESTINAL MORPHOLOGY

The operative procedure by itself had no apparent influence on the morphology of the intestine. Instillation of PBS (with or without LPC or Triton X-100) caused a slight distention of the loop and a flattening of the mucosa with broadening of the villi. No apparent infiltration of inflammatory cells was found in the mucosa after either treatment. Phosphate buffered saline alone had no evident effect on epithelial morphology as seen by light microscopy (Fig. 3) or by transmission electron microscopy. On the other hand, instillation of LPC or Triton X-100 caused profound changes of the epithelium along most of the villous tips (Fig. 3). These changes included loss of columnar characteristics and varying degrees of karyorrhexis and

Fig. 1 Influence of 20 mM LPC (left) and 1% Triton X-100 (right) on intestinal permeability to FITC-dextran 3000 (●) and 10 000 (■). Permeability in the absence of LPC and Triton X-100, respectively, is indicated by open symbols ((○) FITC-dextran 3000, (□) FITC-dextran 10 000). The ordinate shows the blood concentration at different times after deposition of 3.3 mM FITC-dextran in the distal ileum. Mean of five experiments, vertical bars indicate standard error of the mean.

Fig. 2 Intestinal permeability to FITC-dextran 70 000 (left) and bovine serum albumin (right) in the absence (○) and presence (●) of 20 mM LPC. The ordinate shows the serum concentration at different times after deposition of a mixture of FITC-dextran 70 000 (50 mg/ml) and bovine serum albumin (50 mg/ml) in the distal ileum. Mean of 6–10 experiments; vertical bars indicate standard error of the mean.

karyolysis. Furthermore, both partial desquamation with cells still sticking to the remaining covering epithelium and total desquamation were seen. When the degree of morphological changes in the villous tips was quantitatively assessed, the epithelial damage after instillation of Triton X-100 was found to be more pronounced than that after instillation of LPC (Table).

Ultrastructurally, the above findings were verified. A reduction in number of microvilli,

swelling of mitochondria and cytoplasmic vesiculation were noted after treatment with either LPC (Fig. 4a) or Triton X-100. Furthermore, Triton X-100 caused more pronounced damage with total epithelial desquamation of many villous tips (Fig. 4b). Tight junctions were sometimes opened up between clearly degenerating cells still sticking to the basal membrane, but not between normal or only slightly damaged cells.

INFLUENCE ON MUCOSAL CELLS IN VITRO

The influence of LPC on activities of alkaline phosphatase, 5'-nucleotidase, and N-acetyl-β-glucosaminidase from isolated mucosal cells *in vitro* is illustrated in Figure 5. Thus, mixtures of mucosal cells and increasing LPC concentrations showed increased enzyme activity: the higher the LPC concentration, the higher the enzyme activity. In the presence of 1 mM LPC, the N-acetyl-β-glucosaminidase was increased 4.8 times over that attained in the absence of LPC, whereas corresponding values for alkaline phosphatase and 5'-nucleotidase were 1.7 and 1.5, respectively. By contrast, there was no significant influence of 1 μM–10 mM LPC on the N-acetyl-β-glucosaminidase in a 30 000 g supernatant fraction of disintegrated mucosal cells.

Discussion

Altered intestinal permeability to food constituents

Table Quantitative assessment of the influence of LPC and Triton X-100 on intestinal morphology. The Table shows the percentage of villous tips showing different degrees of morphological changes after exposure to the various agents. Moderate changes include loss of columnar characteristics, karyorrhexis, karyolysis or partial desquamation, whereas severe changes represent total desquamation of epithelium leaving some areas of the basal membrane denuded. A mean of 36 villi (range 22–69) were examined in each intestinal circumference. Values are mean $\pm$ SEM

Agent	Circumferences counted (no)	Morphological changes		
		None	Moderate	Severe
None	10	95.0 $\pm$ 1.5	5.0 $\pm$ 1.5	
PBS	10	91.0 $\pm$ 4.4	9.0 $\pm$ 4.4	
LPC (20 mM)	10	11.7 $\pm$ 3.0	80.9 $\pm$ 2.8	7.5 $\pm$ 3.5
Triton X-100 (1%)	9	1.2 $\pm$ 0.9*	59.6 $\pm$ 7.4*	39.2 $\pm$ 7.7†

* $p < 0.01$ vs LPC treatment. † $p < 0.001$ vs LPC treatment.

Fig. 3 Photomicrographs of intestinal villi exposed to (a) PBS, (b) 20 mM LPC, and (c) 1% Triton X-100. (Weigert's haematoxylin-eosin; 570). In (a) the villus is covered with regular columnar epithelium whereas in (b) the epithelium is degenerated and partially desquamated – that is, some cells are still sticking to the remaining epithelium. A total desquamation of epithelium with denuded basal membrane is seen in (c) (arrows). (The dilated capillaries under the basal membrane of villous tips in (a) and (b) are due to the perfusion fixation).

Fig. 4 TEM micrographs from villous tips exposed to (a) 20 mM LPC, and (b) 1% Triton X-100. Note in (a) severely damaged enterocytes with distorted microvilli (M), numerous cytoplasmic vacuoles, and dilated mitochondria with disrupted cristae. At the bottom of (a) are seen a nucleus (N) and the basal membrane (BM). In (b), denuded basal membrane (BM) is seen. Note also in (b) dilated capillary (C) beneath basal membrane (effect of perfusion fixation).

Fig. 5 Activities of alkaline phosphatase (■), 5'-nucleotidase (●), and N-acetyl-β-glucosaminidase (▲) in mixtures of rat intestinal mucosal cells and different LPC concentrations. The ordinate shows enzyme activity relative to the activity in the absence of LPC. Mean of three experiments; vertical bars indicate standard error of the mean.

and microbial products may underlie the pathogenesis of a variety of diseases. Accordingly, increased absorption of macromolecules has been claimed to occur in coeliac disease, chronic inflammatory bowel disease, IgA deficiency, food allergy, various liver diseases, and others.¹¹⁻¹⁵ Little is known, however, about the mechanisms by which intestinal uptake of macromolecules might be facilitated in these disease states. Although certain bile acids,^{2 16 17} synthetic detergents,¹⁸ and traumatic injury¹⁹ all have been shown to increase gut permeability to macromolecules in various experimental systems, their role in the development of gastrointestinal disease has not been established. Analogously, we recently found that LPC increased the permeability to dextran 3000,³ but the biological significance of this finding is as yet unclear.

The present study shows that LPC may increase the intestinal permeability not only to dextran 3000, but also to dextran 10 000 and, indeed, 70 000. It also shows that LPC may augment the intestinal absorption into blood of bovine serum albumin, a 70 000 dalton protein (Fig. 2). Moreover, LPC apparently released considerable amounts of lysosomal enzyme from mucosal cells *in vitro* (Fig. 5), and increased the shedding of enterocytes from villous tips *in vivo* (Fig. 3). These findings indicate that LPC might damage mucosal cells and impair the intestinal barrier function in the distal part of the

ileum. As a consequence, the intestinal mucosa may allow permeation of potentially antigenic, toxic or carcinogenic material.

The mechanism by which LPC may damage the intestinal mucosa has not been elucidated. We found, however, that much the same or even greater influence than LPC on mucosal structure and permeability was obtained with Triton X-100, a non-ionic detergent. It can be hypothesised, therefore, that the observed effects of LPC can be attributed to its detergent properties. Detergents can alter gut permeability²⁰ and damage cell membranes²¹ although the exact mechanism by which they interact with membranes is only incompletely understood. If LPC-protein interactions are disregarded, increasing LPC concentrations in the phospholipid bilayer will lead to either a generally enhanced membrane permeability by increased membrane 'fluidity', or to the formation of actual pores.²² Also partial micellisation and solubilisation of membrane lipids have to be considered. Both reactions will finally lead to free ion permeation and subsequent osmotic rupture of the cell. Analogously, if the binding affinity of LPC to some integral membrane proteins are high, the accumulation of lysolipid around proteins might result in an extraction of proteins into micelles and, again, formation of 'pores' in the membranes.²² This could explain why LPC 'released' N-acetyl-β-glucosaminidase, a membrane-bounded lysosomal enzyme from the mucosal cells, but had little influence on brush border enzymes such as alkaline phosphatase and 5'-nucleotidase (Fig. 5).

We have thus obtained some evidence to indicate that LPC, a naturally occurring detergent, may impair the mucosal barrier in the rat ileum and so facilitate the intestinal transmission of macromolecules. The relevance of this finding to the development of human disease states remains to be established. Ammon *et al*²³ recently showed that LPC affects rat intestinal transport in the same way as bile acids, fatty acids and synthetic cationic or nonionic detergents, and argued, by comparison with the response of the human jejunum to taurodeoxycholate, that it is likely that LPC generated during the normal process of digestion has a significant influence on intestinal transport in the postprandial phase under physiological conditions. The postprandial concentrations of LPC in the human jejunum range from 2 mM to 4 mM^{24 25} whereas little is known about LPC concentrations in the distal part of the small intestine. It cannot be ruled out, however, that higher concentrations of LPC might occur, particularly if the mucosa is less capable than normal to remove LPC. We recently found that the activity of lysophospholipase was

decreased in ileal mucosa of patients with Crohn's disease and suggested that the mucosa could be less well equipped to handle LPC and, therefore, predisposed for mucosal injury induced by LPC.²⁶ This possibility should not be entirely overlooked because LPC is generated in inflammatory foci,²⁷ has a range of cytotoxic and non-cytotoxic effects on diverse cells²⁸⁻³² and, as shown in this investigation, may enhance the mucosal permeability to potentially antigenic and toxic molecules.

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