Increased absorption of polyethylene glycol 600 deposited in the colon in active ulcerative colitis

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Abstract
A defect in the barrier function of the intestinal mucosa has been proposed as important in both the pathogenesis and systemic manifestations of inflammatory bowel disease. After colonoscopy, polymers of polyethylene glycol (PEG) with molecular weights of 414–810 (mean 600), were instilled in the descending colon of patients with ulcerative colitis (n=17) and in controls without intestinal inflammation (n=8). The patients with active ulcerative colitis (n=6) had a significantly increased uptake of PEGs in the molecular weight range 458–810, measured as urinary excretion over the first 6 hours after instillation. The median values for their excretion were 2.85–3.80% of PEGs instilled compared with 0.32–0.94% for patients in remission (n=11) (p<0.05–0.01) and 0.17–0.60% for the controls (p<0.05–0.01). The differences in absorption of PEG 414 did not reach the preset level of statistical significance. There was a positive correlation between PEG absorption and the endoscopic and histological grading of inflammatory activity in the sigmoid colon (p<0.01–0.001). These findings support a correlation between the presence of active inflammation and PEG absorption. There was little evidence to support the presence of a primary defect in the colonic barrier in patients with ulcerative colitis.

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A defect in the barrier function of the intestinal mucosa has been proposed as an important factor in the pathogenesis of chronic inflammatory bowel disease.1–10
Assessment of intestinal permeability in ulcerative colitis after the ingestion of various markers has yielded conflicting results.11–16 An intrinsic colonic permeability defect has been proposed in ulcerative colitis, regardless of disease activity, after studies with 99mTc-technetium-diethylene-triamino-pentaacetic acid (99mTc-DTPA) and 51 chromium-ethylenediaminetetra-acetic acid (51Cr-EDTA).15–17 In the latter study, excretion of ingested 51Cr-EDTA was increased in active and extensive colitis, although not in quiescent and distal disease.17 The absorption of ingested polyethylene glycols (PEGs) has been found to be normal in patients with active ulcerative colitis.18–20 In three other studies using 51Cr-EDTA, no change in intestinal permeability could be shown in patients with ulcerative colitis when compared with controls.20–24 Oral permeability tests are more likely to reflect small intestinal permeability,20,21 and oral sugar markers may be degraded by colonic bacteria before they cross the mucosa2 rendering them less suitable for assessing colonic permeability. Besides this, there is little, if any, evidence of small bowel pathology in ulcerative colitis, which makes the use of oral markers in this context questionable.

Regional studies seem more appropriate if one is to assess colonic permeability.19 Three studies on regional intestinal permeability in patients with ulcerative colitis have been published. In two, a tendency towards lower uptakes was noted in patients with ulcerative colitis compared with controls after the rectal deposition of sodium 131iodide,25,26 phenolsulphonphthalein, and sulfisoxazole diethanolamine.26 An increased uptake of 51Cr-EDTA was shown in active disease, returning towards normal during remission.3 These data on colonic permeability are limited and have not been correlated to the degree of inflammation present. There are, however, experimental studies suggesting a relationship between active intestinal inflammation and permeability.25,26

In the present study, we investigated colonic permeability and its relationship to the presence of inflammation by measuring the absorption of PEG 600 after intracolonic deposition following colonoscopy in patients with ulcerative colitis and in controls.

Methods

ENDOSCOPY
At colonoscopy the macroscopic appearance of the mucosa in the splenic flexure, the sigmoid colon, and the rectum was graded according to a modified Baron scale27,28: grade 0=normal vascular pattern; grade 1=loss of vascular pattern, mucosal oedema but no bleeding; grade 2=granularity, friability, mucosal bleeding on instrument contact; and grade 3=discrete ulcerations and spontaneous bleeding – that is free blood in lumen when introducing the endoscope.

HISTOLOGICAL GRADING
Biopsy specimens taken a few cm distal to the splenic flexure and in the sigmoid colon were stained with haematoxylin and eosin and were assessed blindly for the degree of inflammation.
We used a four grade scale, originally described by Watts et al29 and slightly modified to reflect the intensity of the inflammation present. Grade 1=normal appearance with intact epithelium, small numbers of lymphocytes, plasma cells, and macrophages in the lamina propria; grade 2=intact epithelium but increased numbers of chronic inflammatory cells in the lamina propria, occasional foci of polymorpho-
nuclear leukocytes; grade 3 = mild epithelial changes, leukocytes within the epithelium or the crypts and ducts, pronounced inflammatory cellular infiltration in the lamina propria; grade 4 = severe inflammatory changes with evidence of crypt abscesses with destruction, inflammatory erosions, or frank ulceration.

Grade 2 was not interpreted as a sign of active colitis, since it has been found in about one third of patients with a normal macroscopic mucosa and no history of colitis.26

PEG 600 COLONIC ABSORPTION TEST

Polymers of ethylene glycol with a molecular weight averaging 600 (PEG 600; HO-(CH2)\textsubscript{n}CH\textsubscript{2}OH, n = 9–18; supplied as Macrogol from Apoteksbolaget, Sweden) were used as a marker of intestinal permeability. After colonoscopy, 3.6 g of PEG in 30 ml water were passed through the working channel of the colonoscope and deposited in the descending colon just below the splenic flexure. The working channel was flushed thereafter with 30 ml sterile water. The procedure was performed with the patient in the left lateral position, which was retained for at least 10 minutes. The patient was thereafter allowed to move about freely apart from lying on the right side. Urine was collected over the first 6 hours after PEG deposition. After measuring the volume, two aliquots of 10 ml each were frozen and stored at −20°C pending analysis. All urine samples were analysed on one occasion.

ANALYSIS

A modification of a previously described reversed phase high performance liquid chromatography technique30 was used for the analyses. Five ml urine were mixed with 5 ml deionised water and 10 ml methanol, and then applied to kieselguhr (Extrelut, E Merck, Darmstadt, Germany) without filtering. Elution was carried out with chloroform (30 ml) and the entire eluate was evaporated under air at 60°C. Two ml of ethanol-water (40:60) were added to the dried residue. The test tube was then put in an ultrasound bath for 5 minutes.

The amounts of PEGs were determined in the extracted samples by reversed phase high performance liquid chromatography. The individual PEG species were identified in the chromatogram by comparing their retention times with the retention time for a pure PEG 634 standard. A sample from the original PEG batch given to the patients was analysed at the same time. Absorption of the various PEGs was expressed as the urinary excretion as a percentage of total dose (3.6 g) given. To describe size selective absorption from the intestinal lumen, a recovery ratio (PEG 766/458) was calculated by dividing the recovery of a large PEG molecule (PEG 766) with the recovery of a smaller PEG molecule (PEG 458).

The originally described analytical method has a variation coefficient of 2–4% in 5% and a recovery of 102±0.3% (mean (SD)).28

Patients

ULCERATIVE COLITIS PATIENTS

Seventeen patients, 10 men and seven women, with ulcerative colitis were investigated. The median age was 42 (range 18–64) years. The median disease duration was 8 (range 0.5–33) years. All patients had normal serum creatinine values at the time of investigation. One patient with active disease underwent colectomy 2 months later and developed renal insufficiency because of amyloid deposits 6 months later.

ACTIVE DISEASE

Six patients had active disease as judged by the combination of symptoms requiring steroid treatment and active inflammation seen endoscopically. All six patients had a friable mucosa that bled on instrument contact (grade 2) with at least one of the splenic flexure, sigmoid colon, or rectum. Furthermore, three of these had a spontaneously bleeding mucosa (grade 3) at one or more of these levels. Four patients had total colitis and two patients had extensive disease—that is, reaching the transverse colon but with an upper limit distal to the hepatic flexure. All patients were on maintenance treatment with sulphasalazine (n = 5) or olsalazine (n = 1). Four patients had prednisolone treatment at the time of colonoscopy.

REMISSION

Eleven patients were in remission. Previously performed colonoscopy and/or barium enema had shown that seven had had total colitis, one patient had disease affecting the transverse colon, and three patients distal colitis—that is, the most proximal extension was below the splenic flexure. None of the patients in remission required treatment other than sulphasalazine or olsalazine. One patient had no current treatment at all. All patients in remission had endoscopic grading 0–1.

CONTROL PATIENTS

Eight patients, four men and four women, with a median age of 64 (range 44–67) years, underwent colonoscopy because of radiologically suspected polyps (n = 3), control after previously removed rectal or colonic polyps (n = 4), or as surveillance after right-sided hemicolectomy due to colonic carcinoma (n = 1). The investigation showed no
abnormalities in any of the control patients. All had an endoscopic grade 0. Three patients had current medication: one had atenolol, one chlorpropamide, and the third took vitamins. All controls had normal serum creatinine concentrations.

ETHICS
The study was approved by the Committee of Research Ethics at the Faculty of Health Sciences, Linköping University. The participants gave their informed consent before participation.

STATISTICAL EVALUATION
Median and interquartile (25th to 75th centiles) range (IQR) values are given for each group, since the data do not conform to a Gaussian distribution. The Kruskal-Wallis and the two tailed Mann-Whitney U test were used for comparison between groups. The Spearman rank order correlation coefficient, rs, was used for correlation between PEG absorption and endoscopic and histological data. Analysis of variance was used for assessing any influence of age on PEG absorption. A probability of less than 5% was accepted as statistically significant (p<0.05).

Results
The median absorption of different PEG fractions varied between 2.85 (IQR 1.37-3.85) and 3.80 (1.86-4.62)% in patients with active ulcerative colitis, between 0.32 (0.20-0.66) and 0.94 (0.31-1.72)% in patients in remission, and between 0.17 (0.07-0.62) and 0.87 (0.70-1.33)% in controls (Figure). With the exception of PEG 414, the differences in absorption were statistically significant when comparing patients with active ulcerative colitis with patients in remission (p<0.05-0.01) and controls (p<0.05-0.01).

When comparing patients in remission with controls, absorptions of the various PEG fractions were similar (p>0.05). There were no differences between any of the groups when comparing the recovery ratios (PEG 766/458).

Maximal endoscopic and histological gradings of inflammation are given in the Table. A positive correlation was found between the endoscopic grading of inflammation in the sigmoid colon and the absorption of PEGs 458-810 (r s 0.58-0.77, p<0.01-0.001) as well as between the histological grading of inflammation in biopsy specimens from the sigmoid colon and absorption of PEGs 502-810 (rs 0.55-0.63, p<0.01-0.001).

Patients with active disease receiving steroids (n=4) did not differ from the active group as a whole: the absorption of PEGs in the range 502-810 in these patients was raised (p<0.05) when compared with patients in remission and controls. Two patients with active disease had no steroids at the time of investigation. One had the highest absorption over the whole PEG range, recovery in the urine varying from 5.43 to 9.13%. The other patient had values (1.37-1.86%) below the medians for the other five patients with active disease, but still higher than those in remission.

In the active group, the lowest excretion (typically below 1%) was found in one patient who later developed renal insufficiency. He had, however, at the time of investigation a normal serum creatinine value.

Patients with active disease and patients in remission were significantly younger than the control patients, p<0.05. The age distributions in patients with active disease and patients in remission were similar (p>0.05). When age was introduced as a concomitant variable, the differences in absorption of PEGs were still significant (p<0.01).

Discussion
This study showed an increased absorption of PEG 600 in active ulcerative colitis, indicating an increased colonic permeability, whereas absorption in patients in remission was similar to that in controls. There was no size selective absorption in any group as judged from the recovery ratios of PEG 766/458.

Although the patients in remission showed a slightly higher absorption of PEGs, this rise was not statistically significant. We found little evidence for the existence of an intrinsic colon permeability defect in ulcerative colitis regardless of disease activity, which has been proposed after studies with oral 99mTc-DTPA and 51Cr-EDTA.10 11 Our findings agree with a previous study that showed an increased absorption in active ulcerative colitis after the colonic administration of 51Cr-EDTA.11 An increased absorption of PEGs has also been shown in Crohn’s colitis, using the same method as in this study, during both active disease and remission.9

The patients with active disease and the patients with disease in remission were younger than the controls. The age distributions of patients with active disease and in remission were, however, very similar and analysis of variance did not show any influence of age on PEG absorption. No change in intestinal permeation ratios with age has been shown using oral double sugar absorption tests.12 13 It is not known whether there is an increase in colonic absorption of PEGs with age in humans such as is seen in rats,13 and whether this could have influenced the results.

An increased absorption of PEGs in active ulcerative colitis could be explained by the presence of inflammation resulting in the loss of mucosal integrity. The importance of active inflammation is underlined by the positive correlation we found between the endoscopic and histological grading of inflammation and PEG
absorption. In Crohn’s disease intestinal permeability for Cr-EDTA has been shown to parallel inflammatory activity measured using labelled leukocytes.14

There are probably other factors also responsible for the differences in permeability observed between patients with and without active disease. It is possible that an increased mucosal blood flow due to the inflammation per se can contribute to increased absorption.15 Ultrastructural changes in colonic mucosa consistent with increased absorption have been described in ulcerative colitis, in both affected and unaffected areas.16 However, there seem to be no morphological studies on the actual uptake of trace substances and their route through the epithelial barrier. Although the kinetics of intestinal absorption of different PEG sizes is not fully known, absorption of PEG 900 is compatible with simple passive diffusion and only minimal transport occurs through the brush border.17 This implies transport through paracellular tight junctions, and disorganisation of tight junctions has been described in Crohn’s disease of the ileal mucosa,18 as has increased uptake of PEG 600 in Crohn’s colitis.19 Furthermore, a change in PEG permeability in the physicochemical properties of the mucus in the colon20 may contribute. The ‘leakiness’ of the colonic epithelial cells in ulcerative colitis shown in vitro,21 is probably of minor importance since PEGs do not cross lipid cell membranes.22

The inflammatory cell infiltrate and its chemical products may influence intestinal permeability in various ways. It has been suggested that increased permeability is the result of changes in the epithelial cell mediated immune response.23 In cultured human intestinal epithelial cell monolayers, the transepithelial resistance fell and the macromolecular permeability increased parallel to the numbers of polymorphonuclear leukocytes that migrated through intercellular occluding junctions.24 In animal experiments, increased mucosal permeability could be prevented by depletion of granulocytes with antineutrophil serum,25,26 and ileal mucosal permeability increased after exposure to neutrophil derived oxidants.26

The role of chemotactic N-formylated peptides in the regulation of mucosal permeability has recently come to light. These peptides are produced by bacteria normally found in the human bowel26 and in patients with ulcerative colitis.27 Formyl-methionyl-leucyl-phenylalanine (FMLP) is the major chemotactic peptide produced by Escherichia coli; N-formylated peptides bind to specific receptors on polymorphonuclear leukocytes28 thereby activating the cells to release such substances as lysosomal enzymes, oxidative metabolites29 and eicosanoids.30 They produce severe colitis after instillation in the colon.31,32 They cause an increase in gut permeability when installed in the small bowel,33 an increase that is abolished after granulocyte depletion using antineutrophil serum.34 Furthermore, FMLP activated neutrophils can evoke histamine release from mast cells,35 and histamine and histamine mono-chloramine enhance ileal permeability.36

Mucosal prostaglandin E2 is raised in ulcerative colitis,37,38 and its possible influence on colonic permeability is not known. In a segmental perfusion model, however, prostaglandin E2 has been shown to decrease small bowel absorption of PEG and water.39

All these data point to the importance of leukocytes in the regulation of intestinal permeability. Indeed it has been proposed that any increase in permeability in patients with inflammatory bowel disease results from polymorphonuclear transmigration through the intestinal mucosa.40

Four of our six patients with active disease were steroid therapy and they still had a greater absorption of PEGs than the patients in remission and controls. This is in agreement with one study comparing patients with and without cortisone and sulphasalazine therapy where no influence on intestinal permeability was shown.37

In conclusion, we have shown an increase in absorption of PEG 600 molecules after deposition in the colon of patients with active ulcerative colitis. Moreover, PEG absorption correlated to the degree of inflammation as observed endoscopically and histologically. These findings indicate that the increased permeability that most probably represents a change in mucosal barrier function depending on the presence of inflammation, and is not the result of a primary epithelial defect.

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Increased absorption of polyethylene glycol 6000 depot in the colon of active ulcerative colitis.


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