Sulphation of colonic and rectal mucin in inflammatory bowel disease: reduced sulphation of rectal mucus in ulcerative colitis

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INTRODUCTION

The adherent mucous layer of the gastrointestinal tract plays a vital physiological role as a lubricant and as a protective barrier against luminal contents [1]. Approximately 1% of the normal human colonic bacterial flora are capable of utilizing mucin as their sole energy source [2] and there are probably many other colonic bacteria in addition that contribute to the faecal pool of mucus-degrading enzymes. There is a continual balance between the synthesis and secretion of mucin and its breakdown by bacterial glycosidases [3, 4]. Colonic mucus glycoprotein is heavily sulphated in contrast to mucin from the stomach or small intestine [5]. Sulphation confers increased charge, which possibly affects the rheological properties of the mucus, but, perhaps more significantly, sulphation of complex carbohydrates confers resistance to their enzymic degradation by glycosidas [6, 7]. Therefore it is reasonable to postulate that defects in colonic mucin sulphation will render the mucin more vulnerable to bacterial degradation and could be a pathogenic mechanism in ulcerative colitis [8].

Histochemical studies have suggested reduced sulphation of colonic mucin in inflammatory bowel disease [9, 10]. However, direct biochemical evidence for a defect in mucosal incorporation of sulphate into mucin in patients with inflammatory bowel disease is lacking.

In this study, colonic biopsies from patients with ulcerative colitis and patients with Crohn's disease were examined in an in vitro culture system for their ability to incorporate radiolabelled sulphate.

METHODS AND MATERIALS

Patients

Colonic biopsies were taken from the rectum of patients with ulcerative colitis (n=9; age 20–69

1. Normal colonic mucin is heavily sulphated and this increases its resistance to degradation by bacterial enzymes. Any defect in mucus sulphation could therefore be important in the pathogenesis of ulcerative colitis.

2. Rectal biopsies taken at colonoscopy from patients with ulcerative colitis (n=9), patients with Crohn's disease (n=6) and control subjects (n=16) were cultured for 24 h in the presence of [35S]acetylglucosamine and [35S]sulphate. Mucin was then extracted and purified, and the ratio of [35S]sulphate to N-[3H]acetylglucosamine incorporated into pure mucin was assessed.

3. The ratio of [35S]sulphate to N-[3H]acetylglucosamine incorporated into mucin in Crohn's disease was higher in the rectal biopsies (P=0.26) but this regional variation was not observed in either ulcerative colitis (rectum: 0.450, 0.262–0.773; right colon: 0.470, 0.321–0.690, P=0.3) or Crohn's disease (rectum: 0.459, 0.260–0.815; right colon: 0.492, 0.260–0.929, P=0.8).

4. There was no significant difference in [35S]acetylglucosamine incorporation among the three groups (control subjects: 21 195, 16 611–32 695 d.p.m./mg of biopsy protein content; ulcerative colitis: 12 108, 7663–21 548 d.p.m./mg of biopsy protein content; Crohn's disease: 14 891, 8620–34 419 d.p.m./mg of biopsy protein content, P=0.17), suggesting that there is a selective reduction of incorporation of sulphate per mucin side chain.

5. This study demonstrates a reduced ability of the rectal mucosa to sulphate mucin in patients with inflammatory bowel disease.
years, median 41 years; three males, six females), patients with Crohn's disease (n=6; age 38–69 years, median 43 years; two males, four females) and from control subjects (iron deficiency anaemia, irritable bowel syndrome and patients with a history of Crohn's disease and 14 control subjects). Patients were classed as active or inactive by the Truelove–Witts criteria for ulcerative colitis [11] and the Harvey–Bradshaw disease activity index for Crohn's disease [12]. Colonoscopic disease activity grading [13] was recorded and histological examination of biopsies from adjacent mucosa was performed. Of the patients with ulcerative colitis, four had macroscopic evidence of active disease as seen at colonoscopy, whereas five had macroscopically normal mucosa. Histological assessment of biopsies taken from adjacent tissue showed significant acute inflammation in four patients and inactive disease in five patients. The patients with Crohn's disease all had ileo-colonic involvement, but only one patient had macroscopic and histological rectal disease.

Ethical approval

The study was approved by the Ethical Committee of the South Sefton Health Authority.

Tissue culture methods

Biopsies were cultured by the method of Browning & Trier [14]. Each biopsy was placed on an alloy mesh and floated on culture medium in an organ culture dish (Becton Dickinson, NJ, U.S.A.) orientated with the epithelial surface upward and incubated for 24 h at 37°C in 95% O₂, 5% CO₂ in a 100% humidified incubator. The culture medium was 90% (w/v) RPMI 1640 and 10% (w/v) foetal calf serum (Gibco, Uxbridge, Middx., U.K.), to which was added 100 μg of gentamicin/ml, 60 μg of nystatin/ml, 5 μCi of N-[3H]acetylglucosamine/ml (New England Nuclear, Boston, MA, U.S.A.) and 5 μCi of sodium [35S]sulphate/ml (New England Nuclear).

Glycoprotein extraction

At the end of the culture period the biopsies were harvested and ultrasonicated in 10 ml of Tris–HCl buffer, pH 8.0 (0.05 mol/l), followed by centrifugation at 110 000 g for 90 min. The supernatant was then dialysed extensively against deionized water, freeze-dried and reconstituted in 400 μl of Tris–HCl buffer, pH 8.0. The culture media containing secreted mucus were centrifuged at 110 000 g for 90 min and the supernatant was treated in the same way. Two hundred microlitres of the solubilized material was then injected via a 200 μl sample loop on to a 10 mm x 300 mm gel filtration column containing monodisperse cross-linked agarose beads (Superose 6; Pharmacia, Uppsala, Sweden). It was eluted with 0.1 mol/l Tris–HCl buffer, pH 8.0, at a flow rate of 0.25 ml/min with continuous monitoring of absorbance at 280 nm and collected in 100 × 0.5 ml samples. The fraction corresponding to the initial absorbance peak had been confirmed as pure mucin by previous work [15]. These fractions were then added to 10 ml of Optiphase-safe (FSA Laboratory Supplies, Loughborough, Leics., U.K.) and the incorporated N-[3H]acetylglucosamine and [35S]sulphate were quantified in a liquid scintillation counter (LKB, Turku, Finland). The protein contents of both purified mucin and crude soluble glycoprotein fractions samples were assayed by a modified Lowry method [16]. Previous studies had shown linear incorporation of sulphur and N-acetylglucosamine throughout the culture period of 24 h [7]. Histological examination showed preserved morphological features in biopsies after 24 h cultures.

Results were expressed as: (i) ratio of d.p.m. of [35S] to d.p.m. of [3H] in purified mucin, (ii) d.p.m./mg of protein in the ultrasonicated biopsy and (ii) d.p.m./mg of pure mucin protein content.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance for between-group comparisons of patients with ulcerative colitis, patients with Crohn's disease and control subjects after logarithmic transformation, and all results are expressed as geometric means and 95% confidence intervals. Individual comparisons were performed by a modified t-test (Welch). Paired data were compared by a paired t-test after logarithmic transformation of the data.

RESULTS

The incorporation of [35S]sulphate into pure mucin by rectal biopsies, as expressed in d.p.m./mg of biopsy protein content, was significantly lower in patients with ulcerative colitis (6980, 3570–13 645 d.p.m./mg, geometric mean, 95% confidence intervals) compared with control subjects (18 705, 12 453–28 095 d.p.m./mg; P < 0.01). In patients with Crohn's disease, incorporation of [35S]sulphate into pure mucin was lower (12 319, 4644–32 677 d.p.m./mg), but the difference did not reach statistical significance (P = 0.17, Fig. 1).

Similar results were obtained when [35S]sulphate incorporation was expressed as d.p.m./mg of pure mucin protein content (control subjects: 299 061, 205 233–435 805 d.p.m./mg; ulcerative colitis: 140 331, 70 756–278 184 d.p.m./mg, P < 0.05; Crohn's disease: 206 913, 139 202–307 737 d.p.m./mg, P = 0.16).

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Mucin sulphation in colitis

P = 0.17

Pt = 0.01

Mucin sulphation in colitis

Fig. 1. Incorporation of [35S]sulphate into purified mucin by cultured rectal biopsies taken from control subjects, patients with ulcerative colitis and patients with Crohn's disease expressed as d.p.m./mg of biopsy protein content.

In the control subjects, in whom both proximal colon and rectal biopsies were obtained (n = 14), the ratio of [35S]sulphate to N-[3H]acetylglucosamine incorporation into mucin was higher in the rectal biopsies (0.882, 0.618–1.022) than their paired proximal colonic biopsies (0.602, 0.421–0.861, P < 0.01, paired signed rank test, Fig. 2), but this regional variation was not observed in either ulcerative colitis (rectum: 0.450, 0.262–0.773; right colon: 0.470, 0.321–0.690, P = 0.3, n = 9) or Crohn's disease (rectum: 0.459, 0.260–0.815; right colon: 0.492, 0.260–0.929, P = 0.8, n = 5).

**DISCUSSION**

The ratio of [35S]sulphate to N-[3H]acetylglucosamine incorporation into mucin was significantly reduced in cultured rectal biopsies taken from patients with ulcerative colitis. It was also reduced in Crohn's disease, but this did not reach...
statistical significance. This finding was not due to differences in N-[3H]acetylglucosamine incorporation and is therefore likely to be due to reduced sulphation of mucus rather than to any overall alteration in mucus synthesis. It is not possible to say whether this defect in sulphur incorporation is a primary or secondary phenomenon, but the finding that it bears little relationship to disease activity, clinically or histologically, would suggest that it is not merely due to mucosal inflammation alone.

These findings are in keeping with histochemical studies using high iron diamine staining characteristics, which showed decreased mucin sulphation in the mucosa of patients with colonic polyps, ulcerative colitis and colonic carcinoma [5].

The degree of sulphation of colonic mucin in the normal colon, as detected by high iron diamine staining, increases from the caecum to the rectum [5, 17]. It is thus of interest to note that, in the present study, the degree of sulphate incorporation was also shown to be higher in the rectum than in the right colon. As bacterial load, and therefore, presumably, mucin-degrading enzyme concentration, rises from the proximal to distal colon, it would be of physiological adaptive advantage for the colonic mucin to increase its degree of sulphation in parallel with this. The present study demonstrates that in both ulcerative colitis and Crohn's disease, there is a lack of this normal increase in sulphation of rectal compared with proximal colonic mucus. In ulcerative colitis and, to a lesser extent, Crohn's disease the overall sulphation of rectal mucus is shown to be reduced compared with control subjects. This is likely to make the mucin more vulnerable to the bacterial mucolytic enzyme load in the left colon and would be in keeping with the fact that ulcerative colitis most typically affects the distal colon.

Whether the reduced rectal mucus sulphation in inflammatory bowel disease is a primary or secondary phenomenon, it is likely that it diminishes the ability of the mucus barrier to defend against penetration of the mucosa by antigenic or toxic material. Sulphation of mucins tends to occur in sites where bacteria are plentiful, e.g. normally in the colon, gallbladder and mouth, and abnormally in the achlorhydric stomach [5], bronchiectatic lung and ileal pouch [18]. The factors which regulate this sulphation (which is performed by sulphotransferases located in the Golgi apparatus [19]) are unknown and are worthy of further study.

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