Alimentary tract and pancreas

Faecal mucus degrading glycosidases in ulcerative colitis and Crohn's disease

J M RHODES, RUTH GALLIMORE, E ELIAS, R N ALLAN, AND J F KENNEDY

From the Departments of Medicine, Queen Elizabeth Hospital and General Hospital, Birmingham and Department of Chemistry, University of Birmingham, Birmingham

SUMMARY Because the normal faecal flora includes bacteria which can produce mucus-digesting glycosidases, it follows that increased digestion of colonic mucus by these bacterial enzymes could be important in the pathogenesis of ulcerative colitis. Faecal activities of potential mucus-degrading glycosidases have therefore been assayed in samples from patients with inflammatory bowel disease and normal controls. The enzymes α -D-galactosidase, β -D-galactosidase, β -Nac-D-glucosaminidase α -L-fucosidase and neuraminidase were assayed. Considerable glycosidase activity was present in most faecal samples. Similar activities of all the enzymes assayed were found in faeces from patients with ulcerative colitis, Crohn's disease and normal controls and there was no significant correlation with disease activity. These results imply that relapse of ulcerative colitis is not initiated by increased degradation of colonic mucus by faecal glycosidases but do not exclude a role for bacterial mucus degradation in the pathogenesis of ulcerative colitis.

Approximately 1% of colonic bacteria have been shown to produce glycosidases capable of digesting mucus.¹ Furthermore almost all faecal degradation of colonic mucus results from the action of these bacterial enzymes.²

It follows that ulcerative colitis might result from increased bacterial degradation of colonic mucus which could in turn leave the colonic mucosa exposed to penetration by toxins or allergens. A previous study showed a tendency for faecal glycosidase activity, expressed per gram wet weight, to be lowered non-specifically in patients with diarrhoea.³ This work did not include assays for neuraminidase, α -L-fucosidase and β -Nac-D-glucosaminidase, however, all of which are relevant to mucus degradation. Neuraminidase (sialidase) activity may be of particular interest as much of the colonic mucus is sialated and the terminal sialic acid residue has to be cleaved by neuraminidase before further enzymatic degradation can occur.⁴ It has also been reported that patients with a variety of diarrhoeal diseases including inflammatory bowel disease have a normal faecal population density of mucin-degrading bacteria⁵ but this was assessed by

degradation of hog gastric mucin which is largely unsialated.⁶

The present study was therefore undertaken to quantify potentially mucus-degrading faecal glycosidases in inflammatory bowel disease and in normal controls.

Methods

PATIENTS

Forty five faecal samples were obtained from 35 patients with ulcerative colitis of varying disease activity and 25 samples from 19 patients with Crohn's disease of varying activity of whom 11 had radiological evidence of ileocolonic disease, six colonic disease only and two ileal disease only. Disease activity was graded as inactive, mild, moderate or severe according to established clinical criteria.^{7,8} Eight of the Crohn's disease samples and 18 of the ulcerative colitis samples were from patients receiving oral prednisolone while four of the Crohn's disease samples and 26 of the ulcerative colitis samples were from patients receiving sulphasalazine. No patients had recently received antibiotic therapy. Faecal samples were also obtained from 18 healthy controls (university students). Three of the ulcerative colitis and three of

Address for correspondence: Dr J M Rhodes, Department of Medicine, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH.

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the Crohn's disease patients were hospital inpatients at the time of the study. The median duration of the attack in patients with active ulcerative colitis was five weeks.

ENZYME ASSAYS

Faecal samples for enzyme assays were collected in 20 ml sterile universal containers and frozen at -20°C within four hours of voiding. Preliminary studies had shown that the faecal enzymes were stable at -20°C for at least four weeks. Assays for neuraminidase (pH 4.6) (acylneuraminyldihydrolase, EC 3.2.1.18), α -D-galactosidase (α -D-galactoside galactohydrolase, EC 3.2.1.22) and β -D-galactosidase (β -D-galactoside galactohydrolase) were performed on 43 ulcerative colitis samples, 21 Crohn's disease samples and 18 normal samples. Assays for β -Nac-D-glucosaminidase (2-acetamido-2 deoxy- β -D-glucoside acetamidodeoxyglucosidase, EC 3.2.1.30) and α -L-fucosidase (α -L-fucoside fucosidase, EC 3.2.1.51) were carried out on 24 ulcerative colitis samples, 25 Crohn's disease samples, and 18 normal control samples and assays for neuraminidase at pH 6.0 were carried out on 32 ulcerative colitis samples, 23 Crohn's disease samples and 16 normal samples. These glycosidases are all potentially mucus-degrading.

Three aliquots, each approximately 0.5 g were taken from each faecal sample, thawed and homogenised in 30 ml acetate buffer using a loose fitting homogeniser. Acetate buffer pH 6.4 was used in the assays for β -Nac-D-glucosaminidase and α -L-fucosidase and acetate buffer pH 4.6 in the assays for α - and β -D-galactosidase. Neuraminidase was assayed at pH 4.6 as we found this to be the optimal pH for *Clostridium perfringens* neuraminidase in this assay system and it was thought that this neuraminidase might be relevant to the pathogenesis of colitis. Neuraminidase assay was also performed at pH 6.0 as this approximates well to faecal pH and the optimal pH for faecal neuraminidase activity. pH curves for the faecal enzymes assayed had shown pH optima ranging from pH 5.8 to 6.4. Faecal α - and β -D-galactosidase were both active over a wide pH range with 50% optimal activity between pH 4.0–7.0 and pH 4.5–6.5 respectively. Faecal neuraminidase, α -L-fucosidase and β -Nac-D-glucosaminidase were active over a narrower pH range, all showing a marked fall off of activity below pH 5.0.

The faecal homogenates were centrifuged at 15 000 g for 30 minutes at 5°C and the resulting pellets weighed. The supernatants were filtered through a series of pre-filters culminating in a 0.22 μm pore diameter filter (Millipore UK) to obtain bacteria-free filtrates. Each enzyme was assayed

fluorimetrically in two aliquots from each filtrate using as substrates 4-methylumbelliferyl (4MeU) sodium N-acetyl neuraminatate, 4-MeU α -D-galactopyranoside, 4 MeU β -D-galactopyranoside, 4-MeU 2-acetamide-2-deoxy- β -D-glucopyranoside and 4-MeU α -L-fucopyranoside (Koch Light).

Assays were carried out at 37°C over 15 minutes, with the exception of the neuraminidase assays which were performed over 30 minutes, and were stopped by the addition of 1 ml 1.35M glycine buffer pH 10.7. Fluorescence was estimated using an Aminco-Bowman spectrophotometer at 365 nm extinction, 450 nm absorbance and compared with a blank containing the same substrate added to a previously boiled aliquot of faecal filtrate. Each result represents the mean of six readings. Mean variation between aliquots of the same faecal sample was 13% for neuraminidase, 9% for α -D-galactosidase, 9% for β -D-galactosidase, 7% for β -Nac-D-glucosaminidase and 7% for α -L-fucosidase. Enzyme activity was expressed as μmols methyl umbelliferone released/minute (IU)/g pellet weight. This correction for pellet weight was used to avoid any alterations in enzyme activity that might result simply from a dilutional effect of diarrhoea.

STATISTICAL METHODS

Wilcoxon's rank sum tests were used to compare the enzyme activities obtained in the normal controls with those obtained in ulcerative colitis and Crohn's disease. Kruskal-Wallis one way analysis of variance by ranks was used to compare enzyme activities in patients with ulcerative colitis of differing severity.

Results

Neuraminidase, β -D-galactosidase and β -Nac-D-glucosaminidase activity were detectable in all the faecal samples tested. The other enzymes assayed were also detectable in the majority of samples: α -D-galactosidase in 16 of 18 normal samples, 37 of 43 ulcerative colitis samples and 18 of 21 Crohn's disease samples and α -L-fucosidase in all 18 normal samples, all 24 ulcerative colitis samples and 20 of 21 Crohn's disease samples. Faecal samples that contained a high level of activity of one of the glycosidases almost always contained high levels of activity of the other glycosidases assayed.

There was no significant difference in the level of faecal activity of any of the enzymes tested between normal subjects, patients with ulcerative colitis and patients with Crohn's disease (Table). There was, however, a trend towards increased faecal neuraminidase activity assayed at pH 4.6 in more severe ulcerative colitis (Fig. 1) ($p < 0.1 > 0.05$). This

Table Faecal glycosidase activity

	Neuraminidase pH 4.6	Neuraminidase pH 6.0	α -D- galactosidase	β -D- galactosidase	β -NAc-D- glucosaminidase	α -L-fucosidase
Normals	0.45 $\pm$ 0.39	1.12 $\pm$ 0.65	0.37 $\pm$ 0.63	1.46 $\pm$ 1.38	1.96 $\pm$ 1.32	0.26 $\pm$ 0.29
Ulcerative colitis	0.74 $\pm$ 0.93	1.41 $\pm$ 0.98	0.23 $\pm$ 0.42	0.67 $\pm$ 0.92	2.16 $\pm$ 2.68	0.44 $\pm$ 0.44
Crohn's disease	0.74 $\pm$ 0.77	2.44 $\pm$ 1.36	0.35 $\pm$ 0.92	0.78 $\pm$ 0.97	1.41 $\pm$ 1.54	0.34 $\pm$ 0.71

(IU/g mean $\pm$ sd)

was not demonstrable for faecal neuraminidase assayed at pH 6.0 (Fig. 2). None of the other glycosidases assayed showed any correlation with disease activity in either ulcerative colitis or Crohn's disease. Similar faecal glycosidase activity was found in samples from patients receiving sulphasalazine, corticosteroids, or no treatment.

Discussion

This study confirms that normal faeces usually contain glycosidases that are potentially mucus-degrading. Similar levels of activity of these enzymes are present in the faeces of patients with ulcerative colitis or Crohn's disease and, with the possible exception of neuraminidase, there is no correlation between the level of activity of any enzyme and disease activity.

This is at variance with a previous study which showed a non-specific reduction in faecal glycosidase activity in diarrhoeal diseases³ but in that study enzyme activity was expressed in units per

gram wet weight rather than per gram pellet weight as in the present study and the increased faecal water associated with diarrhoea is likely to have lowered faecal enzyme concentrations expressed in that way.

In ulcerative colitis the trend towards increased faecal neuraminidase assayed at pH 4.6 in active disease is interesting. The pH optimum of faecal neuraminidase in our assay was 6.4 and it is possible that some of the neuraminidase assayed at pH 4.6 is of lysosomal origin, perhaps released by inflammatory leucocytes⁹ or sloughed epithelial cells. We have found, however, that pH 4.6 is the optimum pH for *Clostridium perfringens* neuraminidase in our assay and suspect that most of the neuraminidase assayed even at this pH is of bacterial origin. This is supported by the lack of any increase in active colitis of β -D-galactosidase assayed at pH 4.6 as this enzyme is also potentially lysosomal in origin. There was, however, a surprising lack of increase in active ulcerative colitis of faecal neuraminidase assayed at pH 6.0 and this is

Fig. 1 Faecal neuraminidase (sialidase) assayed at pH 4.6 in normal controls, ulcerative colitis and Crohn's disease. ○ = Patients receiving sulphasalazine therapy. □ = Patients with Crohn's disease affecting ileum only. ● = All other subjects.

Fig. 2 Faecal neuraminidase (sialidase) assayed at pH 6.0 in normal controls, ulcerative colitis and Crohn's disease. Symbols as in Fig. 1.

probably more relevant to the hypothesis because pH 6.0 approximates more closely to the pH of faeces.

The enzymes shown in this study to cleave artificial fluorescent substrates might not necessarily be able to act on mucus glycoproteins. It is likely that they can, however, because all the β -D-glycosidases so far isolated from enteric bacteria have proved active against both artificial and natural substrates.^{10 11}

Much of the colonic mucus is normally sulphated and it is possible that these groups may inhibit degradation of the carbohydrate chains. Faecal sulphatase activity might therefore act synergistically with glycosidase activity to degrade mucus. Faecal sulphatase activity, however, has been shown not to be significantly increased in active ulcerative colitis.¹² Faecal protease activity could lead to breakdown of the polymeric structure of mucins and would also be relevant to colonic disease.

Recently Podolsky and Isselbacher have shown that a group of sialated colonic mucins defined by

ion-exchange chromatography is consistently depleted in ulcerative colitis even in apparently uninvolved mucosa.¹³ In view of the presence of considerable neuraminidase (sialidase) activity in faeces, a hypothesis can be proposed that in ulcerative colitis the colonic mucus may be inherently susceptible to bacterial degradation perhaps as a result of the absence of a mucin type that is normally particularly resistant to desialation. Relapse of colitis could then occur if toxins or allergens were able to penetrate the colonic mucosa as a result of a weakened mucus barrier. Our failure to find any increase in faecal mucus degrading glycosidase activity in ulcerative colitis is still compatible with this hypothesis if one assumes that two coincidentally occurring factors, weakened colonic mucus, and the presence in faeces of an allergen or toxin, are required to initiate a relapse.

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