Faecal sulphatase in health and in inflammatory bowel disease

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SUMMARY Histochemical studies have shown a relative depletion of colonic sulphated mucins (sulphomucins) in active ulcerative colitis. One possible explanation for this could be desulphation by bacterial sulphatases. Studies have therefore been done to determine whether normal faeces contain sulphatase and if so to determine whether this activity is increased in ulcerative colitis. Using a fluorimetric assay considerable sulphatase activity (>0-3 IU/g pellet weight) was found in bacteria free filtrates of the homogenates of nine of 17 faecal samples from healthy controls. This sulphatase activity had an alkaline pH optimum (pH 8.5-9.5). A similar range of faecal sulphatase activity with a similar pH optimum was found in samples from patients with ulcerative colitis (n=39) and Crohn's disease (n=17) and there was no correlation with disease activity in either disease. This faecal sulphatase activity may be involved in the degradation of colonic mucus and merits further study but these findings do not explain the relative depletion of colonic mucosal sulphomucins in ulcerative colitis.

In health a large proportion of the human colonic mucus is sulphated but in active ulcerative colitis and Crohn's disease histochemical studies have shown a relative depletion of colonic sulphomucins. The normal colonic flora contains bacteria which produce glycosidases capable of degrading colonic mucins but it is not known whether sulphatase producing bacteria are also present. It is quite likely that they are as rat faeces have been shown to contain sulphatase activity which is abolished by prior antibiotic treatment and which seems to be produced mainly by a lactobacillus.

There are several reasons why sulphatase production by faecal organisms might be important in the pathogenesis of colonic disease. If the sulphatases were capable of desulphating colonic mucus they would alter its charge and hence its physical properties and also probably increase its susceptibility to further degradation by bacterial glycosidases. Sulphatases might also activate toxins such as lithocholic acid which are detoxified by sulphation. Assays of faecal sulphatase activity have therefore been undertaken on samples obtained from patients with inflammatory bowel disease and from healthy controls.

Methods

SUBJECTS A faecal sample was obtained from each of 17 healthy control subjects (university students) and 39 samples were obtained from 32 patients with ulcerative colitis and 17 samples from 14 patients with Crohn's disease. Of the ulcerative colitis samples seven were from patients in remission, six from patients with mild disease, 15 moderate and 11 severe disease as defined by clinical criteria. Of the Crohn's disease samples four were from patients in remission, four from patients with mild disease, four moderate, and five severe disease judged by similar clinical criteria. None of the Crohn's disease patients had ileo-colonic disease, four had colonic disease only and one had ileal disease only. Only six of the patients with ulcerative colitis and three of the patients with Crohn's disease were hospital inpatients at the time of the study. No patients had received antibiotics within the previous month but 21 of the ulcerative colitis samples and three of the Crohn's disease samples were collected from patients receiving sulphasalazine. The faecal samples were collected in 20 ml sterile universal containers and frozen at -20°C within four hours of voiding. Preliminary studies have shown that faecal sulphatase is stable at -20°C for at least four weeks.

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ENZYME ASSAYS
Three aliquots, each approximately 0.5 g were taken from each faecal sample, thawed and homogenised in 30 ml 0.2M Tris acetate buffer using a loose fitting glass homogeniser. Assays were performed on each sample using Tris acetate buffer at pH's 5.5, 6.5, 7.5 and 8.5. The faecal homogenates were then centrifuged at 15 000 g for 30 minutes at 5°C. The resulting pellets were weighed and the supernatants filtered through a series of prefilters culminating in a 0.22 μm pore diameter filter (Millipore, UK) to obtain a bacteria free filtrate. Two aliquots of each filtrate were assayed fluorimetrically for sulphatase activity using 0.02 mM 4-methylumbelliferyl sulphate (Sigma) as substrate. Assays were performed over 10 minutes at 37°C, inhibited by the addition of 0.1 ml 1·33 M glycine buffer pH 10.7 and fluorescence estimated immediately using an Aminco–Bowman spectrophotometer at 365 nm extinction, 450 nm absorbance. For each sample the results obtained at each pH represent the mean of six readings. Mean variation between aliquots from the same faecal sample was 4%. Enzyme activity was expressed as μmol methyl umbellifere released/minute (IU)/g pellet weight. The results were expressed in this way to avoid misleading alterations in enzyme concentration that might otherwise result from a dilutional effect of diarrhoea.

Results
Sulphatase activity >0.3 IU/g pellet weight was detectable at pH 8.5 in nine of 17 normal faecal samples, 12 of 39 ulcerative colitis samples and six of 17 Crohn's disease samples. In all samples tested from control subjects and patients with ulcerative colitis or Crohn's disease the faecal sulphatase had an alkaline pH optimum (pH 8.5–9.5) (Fig. 1). There was no significant difference overall between faecal sulphatase activity in samples obtained from the normal controls and the samples obtained from patients with ulcerative colitis or Crohn's disease. (Fig. 2, Table). Although several high faecal sulphatase values were recorded in patients with active ulcerative colitis there was no obvious trend towards increased faecal sulphatase in more active disease in either ulcerative colitis or Crohn's disease. Little or no sulphatase activity was detectable in any of the samples tested at pH 5.5 or 6.5 (Table 1). In ulcerative colitis there was no significant difference in faecal sulphatase activity between those patients receiving sulphasalazine (0.75±1.97 IU/g at pH 8.5, mean±SD) and those not (1.16±2.19 IU/g at pH 8.5, mean±SD).

Discussion
This study shows that sulphatase activity is commonly present in normal human faeces. The sulphatase has an alkaline pH optimum (between pH 8.5 and pH 9.5) which makes it probable that it is bacterial in origin, like the rat faecal sulphatase previously reported, as the lysosomal sulphatase

<table>
<thead>
<tr>
<th>pH 5.5 IU/g</th>
<th>pH 6.5 IU/g</th>
<th>pH 7.5 IU/g</th>
<th>pH 8.5 IU/g</th>
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<tbody>
<tr>
<td>Median</td>
<td>Range</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>Normal subjects (n=17) 0.00</td>
<td>0.00-0.02</td>
<td>0.02</td>
<td>0.00-0.21</td>
</tr>
<tr>
<td>UC inactive (n=7) 0.00</td>
<td>0.00-0.07</td>
<td>0.01</td>
<td>0.00-0.16</td>
</tr>
<tr>
<td>Mild (n=6) 0.01</td>
<td>0.00-0.09</td>
<td>0.10</td>
<td>0.00-0.24</td>
</tr>
<tr>
<td>Moderate (n=15) 0.00</td>
<td>0.00-0.06</td>
<td>0.02</td>
<td>0.00-0.28</td>
</tr>
<tr>
<td>Severe (n=11) 0.00</td>
<td>0.00-0.14</td>
<td>0.00</td>
<td>0.00-0.45</td>
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<tr>
<td>CD inactive (n=4) 0.01</td>
<td>0.00-0.04</td>
<td>0.05</td>
<td>0.00-0.16</td>
</tr>
<tr>
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</tr>
<tr>
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<td>0.00-0.13</td>
</tr>
<tr>
<td>Severe (n=5) 0.00</td>
<td>0.00-0.03</td>
<td>0.00</td>
<td>0.00-0.07</td>
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**Fig. 2** Faecal sulphatase assayed at pH 8.5 in normal controls and patients with ulcerative colitis and Crohn's disease.

арилсуфатазы A и B имеют кислые значения pH оптимума, так же как и человеческая N-акетил глюкозамин 6-сульфат суффатаза и N-акетил галактозамин 6-сульфат суффатаза, тогда как миросомальная арилсуфатаза C нерастворима и редко обнаруживается в тканях.12 Важно отметить, что многие из этих суффатаз имеют нейтральную или щелочную pH оптимумы.13

Суффатазы, способные действовать на 4-меанфелин сульфат в качестве субстрата, использованного в нашем исследовании, возможно, не обязательно способны отщеплять сульфат группы из колонного слизи, хотя было установлено, что некоторые из них способны делать это, если кишечные биопсии были выращены в экстрактах кала, аналогичных тем, что использовались в этом исследовании.14

В то время как pH оптимум суммы фекальных суффатаз выше нормального или колитического фекального pH (5,5–6,5, приближенно), эти гистохимические исследования показали, что фекальные экстракты способны отщеплять сульфат от колонного слизи при pH 5,5. Это также известно, что некоторые бактерии способны образовывать суффатазы, которые отщепляют сульфат литочоликовой кислоты,15 что потенциально вредно для колонного слизи.

В этом исследовании были обнаружены сходные уровни суффатазной активности в фекалиях здоровых контролей и у пациентов с умеренным и активным колитом. Хотя небольшое количество образцов от пациентов с активным колитом содержали значительно большую суффатазную активность.

Изменение сульфатации колонного слизи, выявленное гистохимическим методом в колитическом колите, может быть связано либо с увеличением уязвимости слизи к отщеплению бактериями, либо с уменьшением сульфатации при синтезе слизи.

Действие суффатаз, способных отщеплять слизи и токсинов, обычно вовлекаемых в процесс активации сульфатации, могло бы иметь важное значение в колитических заболеваниях. Этот работы были выполнены с помощью гранта от Birmingham Central District Endowment Fund.

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