Thiol methyltransferase activity in inflammatory bowel disease

W E Roediger, W J Babidge

Abstract

Background—Luminal anionic sulphide may contribute to epithelial damage in ulcerative colitis. Thiol methyltransferase (TMT) governs sulphide detoxification by the colonic mucosa and circulating erythrocytes.

Aims—To measure levels of TMT activity in erythrocytes of surgically treated cases of colitis or in rectal biopsies of defined groups of colitis.

Patients—Venepuncture blood was obtained from 37 blood donors and 27 subjects who had previously undergone a proctocolectomy for colitis: 18 for ulcerative colitis and nine for Crohn’s colitis. Rectal biopsies from 122 cases were obtained: 47 without mucosal disease, 33 post-colon resection for cancer, 14 with moderate to severe ulcerative colitis, 15 with quiescent ulcerative colitis, seven with acute Crohn’s colitis, and six with radiation proctitis.

Methods—TMT activity was measured by high performance liquid chromatography with radioactive detection to measure \(^{14}\)C methylmercaptoethanol formation, the reaction product of cell extracts incubated with mercaptoethanol and \(^{14}\)C S-adenosylmethionine.

Results—Erythrocyte TMT activity of surgically treated cases of colitis was significantly elevated (p<0.001) compared with control cases. TMT activity of rectal biopsies was significantly decreased (p<0.02) in acute but not quiescent ulcerative colitis. No primary defect of TMT activity was found in any case of unoperated colitis but mucosal activity was diminished with disease progression of ulcerative colitis. Studies of genetic control of TMT activity of erythrocytes in inflammatory bowel disease appear worthwhile.

Conclusions—Erythrocyte TMT activity was persistently elevated after proctocolectomy for Crohn’s disease and ulcerative colitis. No primary defect of TMT activity was found in any case of unoperated colitis but mucosal activity was diminished with disease progression of ulcerative colitis. Studies of genetic control of TMT activity of erythrocytes in inflammatory bowel disease appear worthwhile.

Keywords: thiol methyltransferase; hydrogen sulphide; methylation; ulcerative colitis; Crohn’s disease

Bacterially produced hydrogen sulphide may play an intermediary role in the pathogenesis of ulcerative colitis (UC). In support are observations of increased rates of sulphide production from stool samples of colitis sufferers1 2 compared with control cases and increased luminal levels of sulphide in colitis,3 4 although rectal dialysis, a poor reflector of luminal sulphide production, did not show elevated levels of sulphide in colitis.5 Hydrogen sulphide impairs fatty acid oxidation in colonocytes,6 the chief energy substrate of these cells, and with increasing concentration also diminishes glucose oxidation.7 Decreasing the formation of sulphide in the colon by therapy with sulphasalazine,8 9 bismuth salts,10 11 or by decreasing sulphur amino acid intake12 produced treatment benefit for microscopic and UC patients. A low sulphate diet in patients with UC reduced bacterial sulphide formation.13

Despite evidence in support of sulphide in the causation of colitis, sulphide instilled in fractionated doses into the colon did not produce colitis14 although sulphide enhanced epithelial cell turnover in colonic biopsies.15 In contrast, continuous infusion of sulphide produced mucosal damage.16 Sulphide enhanced the oxidative burst of activated immune cells17 and stimulated release of nitric oxide from nitric oxide carriers18 thus placing sulphide in an intermediary category as a damaging agent to the colonic mucosa.

Protection against cellular damage by sulphide results from methylation of sulphide to less toxic methyl derivatives.19 Methylation is governed by a supply of the methyl donating agent S-adenosylmethionine (SAM), the availability of ATP, and the activity of the methylating enzyme thiol methyltransferase (TMT) which is greater in the colonic mucosa than in the liver.20 Circulating red blood cell activity of TMT is elevated in cases of UC compared with controls.21 22 Levels of TMT activity in rectal biopsies of UC have been reported as normal.24-28 The aim of the present study was to measure TMT activity in erythrocytes of cases who had undergone proctocolectomy for colitis and who were off medication for colitis and therefore free of bias from medication. TMT activity was also measured in rectal biopsies in defined groups of colitis where disease activity was carefully documented. The present results were compared with those previously reported.

Patients and methods

PATIENTS

Approval to conduct the study was obtained from the Ethics Committee of the Queen Elizabeth Hospital and University of Adelaide.

Abbreviations used in this paper: CD, Crohn’s disease; SAM, S-adenosylmethionine; TMT, thiol methyltransferase; UC, ulcerative colitis.
For measurement of TMT activity in erythrocytes, patients were recruited from the Ileostomy Association of South Australia and blood samples for comparative purposes were obtained from the Blood Transfusion Services of South Australia, although these subjects did not have a proctocolectomy. Venepuncture samples were obtained in the non-fasting state during the morning and collected in lithium heparin. All UC patients had a total proctocolectomy with a Brooke’s type terminal ileostomy and none was receiving steroids or sulphasalazine. None of the subjects had smoked for at least 10 years. There were 18 patients, 11 females and seven male, aged 36–86 years (mean 60.4 (SEM 3.4)) who had undergone their operation 2–50 (mean 18.4 (SEM 2.9)) years ago. There were nine patients, six females and three males, who had a proctocolectomy and Brooke’s ileostomy for Crohn’s colitis, aged 21–80 (54.8 (6.7)) years, with their operation 6–37 (16.6 (3.7)) years ago. Three of the patients had small bowel resections of less than 30 cm and none was receiving sulphasalazine or sulphasalazine 105 000 g Cr51 for 10 minutes. Plasma was removed and an equal volume of normal saline added. Following gentle mixing, centrifugation at 800 g, the procedure was repeated. After removal of plasma, ice cold distilled water (20 ml) was added and the mixture centrifuged at 13 000 g for 10 minutes. The supernatant was discarded and the pellet washed carefully with 3 ml Tris HCl buffer, pH 7.4. After the final wash, 6 ml of Tris buffer were added before recentrifugation. The pellet was reconstituted with 120 ml of Tris buffer before storage at −80°C. Prior to assay the mixture was sonicated on ice for about 10 seconds until homogeneous.

Colonocytes were prepared as described previously from resected segments of colon uninvolved with pathological change. Dithiothreitol was omitted in the preparation. Colonocytes, after isolation, were washed twice in ice cold distilled water (20 ml) and then homogenised in liquid nitrogen using a mortar and pestle. Colon mucosal biopsies which had been collected into preweighed tubes were kept on ice prior to isolating microsomes. The biopsy weight was calculated and 300 µl of 0.25 M sucrose added. This mixture was homogenised on ice and removed prior to washing the vessel with a further 200 µl of 0.25 M sucrose. This mixture was centrifuged at 12 000 g (4°C) for 15 minutes. The supernatant was removed to another tube which was centrifuged at 105 000 g (4°C) for one hour. The supernatant was removed and the pellet reconstituted in 200 µl Tris buffer prior to freezing at −80°C. The mixture was sonicated as above.

TMT assays were performed on the supernatant fraction of biopsies, colonocyte suspensions, or entire suspensions of erythrocyte membranes. Prior to assay protein concentrations of samples were determined according to the method of Lowry and colleagues. TMT activity was determined by a modification of the method of Pazzinio and Weinshilboum as outlined in detail previously. The HPLC system was not changed but the mobile phase was altered to 20% methanol/80% distilled water with 1.75 ml/l dibutyramine in 20% phosphoric acid, pH 3. This mobile phase

### Table 1: Cases who underwent rectal biopsies for measurement of thiol methyltransferase (TMT) activity

<table>
<thead>
<tr>
<th>Condition</th>
<th>No</th>
<th>Females: Males</th>
<th>Age (mean (SEM))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign colonic conditions</td>
<td>47</td>
<td>23:24</td>
<td>65.3 (1.8)</td>
</tr>
<tr>
<td>Treated colonic cancer</td>
<td>33</td>
<td>18:15</td>
<td>70.7 (1.8)</td>
</tr>
<tr>
<td>Acute ulcerative colitis</td>
<td>14</td>
<td>6:8</td>
<td>44.1 (3.9)</td>
</tr>
<tr>
<td>Quiescent ulcerative colitis</td>
<td>15</td>
<td>6:9</td>
<td>47.9 (6.5)</td>
</tr>
<tr>
<td>Acute Crohn’s colitis</td>
<td>7</td>
<td>2:5</td>
<td>33.4 (3.4)</td>
</tr>
<tr>
<td>Radiation proctitis</td>
<td>6</td>
<td>Males only</td>
<td>73.3 (4.0)</td>
</tr>
</tbody>
</table>
eluted unused 14C SAM as a large peak prior to 14C methylmercaptoethanol and there appeared to be no problems with increased background radioactivity.

TMT activity was calculated as pmol of mercaptoethanol formed. The peak area (µV-seconds) was converted to disintegrations per minute (dpm) after determining a conversion factor by injecting a known amount of 14C SAM and relating area to dpm. The specific activity of SAM was then used to calculate pmol of methylmercaptoethanol formed and results expressed as pmol/hour/mg protein.

**STATISTICS**

Analysis of variance (ANOVA) and Wald’s test were applied in comparing results of multiple groups of patients. The Student’s $t$ test for unpaired samples was also used to test the null hypothesis between groups and $p$ values $<$0.05 were considered significantly different.

**Results**

The within assay coefficient of variation of TMT activity was 7% and between assay was 6.4%, with a minimal detection rate of 2 pmol/hour/mg protein, as previously described.\(^{25}\)

Comparison of TMT activity in isolated colonic epithelial cells with rectal biopsy samples of the same tissue specimen revealed a mean (SEM) value of 277.9 (59.5) (n=11) for isolated cells compared with 293.35 (55.7) (n=11) for biopsies. A linear relationship was found between the two tissue samples (fig 1) with a correlation coefficient of 0.92 ($p<0.001$) between the two modes of tissue sampling. These results indicate that biopsy values closely reflect TMT activity of isolated colonoocytes.

Values of TMT activity of erythrocyte membranes (fig 2) varied in healthy subjects with an intact colon (13–222 pmol/hour/mg; mean (SEM) 120.00 (9.6) (n=37)). Values in colectomised cases of UC varied between 130 and 334.0 pmol/hour/mg (mean (SEM) 237.8 (22.9) (n=9)). Values in inflammatory bowel disease were significantly increased ($p<0.001$) compared with healthy cases that had an intact colon.

Values of TMT activity in rectal biopsies (fig 3) were much higher than in erythrocyte membranes, in agreement with previous findings.\(^{25}\) Mean values for healthy controls were 379.7 (SEM 32.9) (n=46), control colon cancer cases 363 (44.5) (n=33), acute UC 227 (33.7) (n=14), quiescent UC 301.8 (33.2) (n=15), active Crohn’s disease 429 (63.2) (n=7), and active radiation proctitis 406.7 (118.7) pmol/hour/mg protein (n=6). Analysis of variance (ANOVA) using STATA (Stata Statistical Software: release 5, 1997, Stata Corporation Texas, USA) detected no difference between the five groups of benign, malignant, acute UC, quiescent UC, and Crohn’s disease ($F_{4,115}=1.53$, $p=0.21$).
Sulphide detoxification in colitis

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previously been validated with regard to repro-

duction and glutathione transferase activity 19 are

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in unoperated cases of UC has been found to be

elevated in both active and quiescent colitis, the

increase being significant in active UC but not

Crohn’s disease.22 23 Current observations in

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TMT activity of erythrocyte membranes in

unoperated cases of UC29 30 with more recent

studies28 intermediary to these values. The

state of TMT activity does not however entirely reflect the

capacity to methylate as the process is depend-

ent on a supply of SAM which in the assay was

exogenously provided. In UC levels of endog-

enous SAM are low in colonocytes40 and meth-

ylation of DNA, which is also dependent on

endogenous SAM, is low in the mucosa of UC.41

Several lines of research15 42 suggest that bacterial metabolism of sulphur may be a compo-

nent of the disease process of UC. The activity of

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ence of the colon. Studies of genetic control of

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be a worthwhile avenue of exploration.

Discussion

TMT activity in the colonic mucosa has been reported as low or high, with more recent studies intermediary to these values. The method we used to assay TMT activity has previously been validated with regard to reproducibility and coefficient of variance. As the activity of TMT may vary along the length of the intestinal tract a fixed region for biopsy was chosen in which colitis usually manifests. Also of concern was whether biopsies, a composite tissue of immune and epithelial cells, would yield TMT activities reflective of isolated epithelial cells. This was found to be the case in healthy mucosa but remains untested in the inflamed mucosa. Immune cells are tolerant of high levels (>1.5 mM) of hydrogen sulphide and may have considerable capacity to methylate as the process is dependent on a supply of SAM which in the assay was exogenously provided. In UC levels of endogenous SAM are low in colonocytes and methylation of DNA, which is also dependent on endogenous SAM, is low in the mucosa of UC. Several lines of research suggest that bacterial metabolism of sulphur may be a component of the disease process of UC. The activity of TMT in the colonic mucosa remains robust in early colitis without significant change from control cases, but a persistently elevated TMT activity in erythrocytes, shown in this and other studies, supports the view that other factors regulate TMT activity irrespective of the presence of the colon. Studies of genetic control of TMT activity in erythrocytes of colitis would be a worthwhile avenue of exploration.

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