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 14C methylmercaptoethanol formation, the reaction product of cell extracts incubated with mercaptoethanol and 14C 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. Thiol methyltransferase 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.

Conclusions—Erythrocyte TMT activity was consistently elevated after proctocolectomy for Crohn’s disease and ulcerative colitis.

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 sufferers compared with control cases and increased luminal levels of sulphide in colitis, although rectal dialysis, a poor reflector of luminal sulphide production, did not show elevated levels of sulphide in colitis. Hydrogen sulphide impairs fatty acid oxidation in colonocytes, the chief energy substrate of these cells, and with increasing concentration also diminishes glucose oxidation. Decreasing the formation of sulphide in the colon by therapy with sulphasalazine, bismuth salts, or by decreasing sulphur amino acid intake produced treatment benefit for microscopic and UC patients. A low sulphate diet in patients with UC reduced bacterial sulphide formation.

Despite evidence in support of sulphide in the causation of colitis, sulphide instilled in fractionated doses into the colon did not produce colitis although sulphide enhanced epithelial cell turnover in colonic biopsies. In contrast, continuous infusion of sulphide produced mucosal damage. Sulphide enhanced the oxidative burst of activated immune cells and stimulated release of nitric oxide from nitric oxide carriers 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. Methylation is governed by a supply of the methyl donating agent S-adenosylmethionine (SAM), availability of ATP, and the activity of the methylating enzyme thiol methyltransferase (TMT) which is greater in the colonic mucosa than in the liver. Circulating red blood cell activity of TMT is elevated in cases of UC compared with controls. Levels of TMT activity in rectal biopsies of UC have been reported as normal. 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 of Human Research 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.
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>

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 steroids or had active Crohn’s disease. There were 37 control cases, 18 females and 19 males, aged 39–68 (52.8 (1.1)) years and none was receiving steroids or sulphasalazine. Colonic 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.27 TMT activity was determined by a modification of the method of Pazmino and Weinshilboum.26 Blood (20 ml) collected into lithium heparin was centrifuged at 800 g for 10 minutes. Plasma was removed and an equal volume of normal saline added. Following gentle mixing, recentrifugation 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 (4°C) 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 previously6 from resected segments of colon uninvolved with pathological change. Dithiothreitol was omitted in the preparation. Colonocytes, after isolation, were washed twice in 0.1 ml dipotassium hydrogen orthophosphate /1 mM EDTA (pH 7.4) then ground finely in liquid nitrogen using a mortar and pestle. Colonic mucosal biopsies which had been collected into preweighed tubes were kept on ice prior to measuring enzyme activity. Six groups of patients were studied and all had complete colonoscopies (table 1). Ages between the various colitis groups were not significantly different but ages between the control groups (benign and malignant) were significantly different (p<0.001, Student’s t test) from the colitis groups. However, there are no reports to suggest that mucosal TMT activity varies with age. There was no significant difference in the sex distribution in any group. Benign conditions were either haemorrhoids, irritable bowel syndrome, or cases who requested colonoscopy to exclude bowel cancer. Cases of treated colonic cancers had resections more than 12 months previously and were undergoing surveillance colonoscopies. All cases of acute colitis histologically based on the Truelove and Richards criteria7 had moderate to severe colitis and all biopsies were taken from the involved area of mucosa. The proximal extent of the disease varied but all had rectosigmoid involvement. Cases of quiescent colitis were clinically and histologically in remission. Cases of Crohn’s colitis had active disease. All acute colitis cases were receiving steroids and sulphasalazine and quiescent cases sulphasalazine or mesalazine only. Cases of mild to moderate radiation proctitis were men who had received radiation therapy for carcinoma of the prostate at least three years previously.

To compare TMT levels of mucosal biopsies with levels of isolated colonocytes, tissues were collected, as previously described,8 from the same colonic resections and biopsies taken adjacent to where mucosal strips were obtained to prepare isolated colonic epithelial cells. Such a comparison was done because biopsies as a composite tissue may have different TMT activity than isolated colonocytes.

METHODS

Erythrocyte membranes were prepared using a modification of the method of Pazmino and Weinshilboum.26 Blood (20 ml) collected into lithium heparin was centrifuged at 800 g for 10 minutes. Plasma was removed and an equal volume of normal saline added. Following gentle mixing, recentrifugation 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 (4°C) 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.

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eluted unused $^{14}$C SAM as a large peak prior to $^{14}$C 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 $^{14}$C 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.\textsuperscript{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 (39.5) (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 colonocytes.

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.\textsuperscript{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 ($F_{4,115}=1.53$, $p>0.05$).
Sulphide detoxification in colitis

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methodology of the assay was carefully
TMT activity. Apart from such a drawback, the
same was found to be
in unoperated cases of UC22 23
dimensions of variance. 28 As the
steroid oxidation in rat colonocytes: A
unoperated cases of UC
TMT activity in erythrocytes of colitis would
influence of the colon. Studies of genetic control of
in the human colon.

Discussion

TMT activity in the colonic mucosa has been reported as low8 or high,9 10 with more recent studies10 intermediary to these values. The method we used to assay TMT activity has previously been validated with regard to repro-
ducibility and coefficient of variance. 28 As the activity of TMT may vary along the length of the intestinal tract29 30 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.28 This was found to be
in healthy mucosa but remains
untested in the inflamed mucosa. Immune cells are
tolerant of high levels (>1.5 mM) of hydro-

den (p=0.186). Using Wald's test after ANOVA a
difference was found comparing the benign
group with the two UC groups together
(p=0.026). Values for active UC compared
with healthy controls were significantly differ-
ent (p<0.02; Student’s test for unpaired
samples) but no other values were significantly different from healthy controls or cancer
control cases.

Mucosal detoxification processes of sulphur-
duction and glutathione transference activity13 are
diminished in UC, particularly in active
disease. Loss of sulphation was detected by
noting diminished sulphation of paracetamol
in rectal dialysate in vivo14 or in vitro by isolated
colonocytes.15 The diminished glutathione
transference activity was observed in human
UC26 as well as in experimental animal colitis.16
Mucosal detoxification by methylation as now
observed was diminished in moderate to severe
UC but not quiescent colitis suggesting that the
primary process of methylation was not
impaired in our cases of UC. The state of TMT
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TMT activity in erythrocytes membranes 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
operated cases of colitis replicated elevated
TMT activity in erythrocyte membranes seen in
unoperated cases of colitis.27–29 suggesting
that factors causing elevation of TMT activity
are present irrespective of the presence of the
colon. One possible factor in unoperated cases
could be a high intake of protein observed in
UC2 3 and particularly foods rich in sulphur
amino acids such as meat.31 32 Sulphur amino
acids can be a source of sulphur via endog-
енous sulphate production (unpublished ob-
servation) for sulphate reducing bacteria to
produce sulphide,2 colonic levels of which are
implicated in the colon.22 23 Studies of genetic control of
TMT activity in erythrocytes of colitis would
be a worthwhile avenue of exploration.

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