Reduced tissue type plasminogen activator activity of the gastrroduodenal mucosa in peptic ulcer disease

M A Wodziński, K D Bardhan, J T Reilly, P Cooper, F E Preston

Abstract
The gastrroduodenal mucosa has a rich blood supply. An active fibrinolytic system is presumably required to maintain vascular patency, and impairment may result in reduced blood flow, focal tissue necrosis, and peptic ulcerogenesis. Tissue type and urokinase type plasminogen activator activity (expressed as mIU/mg protein) and plasminogen activator inhibitor type–1 antigen were assayed in homogenates of gastric and duodenal biopsy specimens taken from patients with: normal endoscopy (controls) (n=14); active duodenal ulcer (n=21); healed duodenal ulcer (n=12); and active benign gastric ulcer (n=15). In controls mean duodenal tissue type plasminogen activator activity was 4110 and urokinase type plasminogen activator activity was 2760 and urokinase type plasminogen activator 170; plasminogen activator inhibitor type–1 was generally undetectable. At the edge of active duodenal ulcers tissue type plasminogen activator was considerably reduced, 2220 (p<0.001) whereas urokinase type plasminogen activator was raised, 290 (p=0.01). At the edge of active benign gastric ulcers tissue type plasminogen activator was substantially reduced, 1160 (p<0.001) but urokinase type plasminogen activator was unchanged. At the scar of healed duodenal ulcers tissue type plasminogen activator was slightly reduced, 3290, but urokinase type plasminogen activator was increased, 308 (p<0.05). H2 receptor antagonist treatment had little effect on tissue type or urokinase type plasminogen activator activity. Plasminogen activator inhibitor type–1 was increased at the edge of active ulcers (p<0.05) especially when tissue type plasminogen activator activity was low (r=−0.61, p<0.05). These findings are consistent with the hypothesis that impaired fibrinolytic activity may be implicated in peptic ulcerogenesis.

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The focal nature of peptic ulcer disease cannot be wholly accounted for by the traditional concept of the underlying pathophysiology, namely an imbalance of ‘attack’ factors (for example acid) and ‘defence’ mechanisms (for example mucus layer, mucosal resistance) as this would be expected to cause more widespread damage. Vascular occlusion within the mucosa, however, would lead to necrosis and ulceration and may account for focal loss of tissue. The gastrroduodenal mucosa has a rich vascular supply essential for its metabolic requirements; to maintain its patency an active fibrinolytic system is presumably needed. It seems possible that if fibrinolysis is impaired, vascular occlusion may result causing tissue damage. The purpose of this study was to test this hypothesis.

Fibrinolytic activity is regulated by two plasminogen activators: tissue type, which is produced in the vascular endothelium and urokinase type, which originates from macrophages, monocytes and interstitial cells, and can also be produced by intestinal cells. In addition to fibrinolytic activity urokinase type plasminogen activator also participates in tissue remodelling and cell migration.1 Plasminogen activator inhibitor type–1, which arises from the vascular endothelium and platelets, inhibits the effect of tissue type plasminogen activator and to an extent urokinase type plasminogen activator.2

Fibrinolytic activity was originally shown in emulsified gastric and duodenal mucosa by a fibrin plate method3 (and a technical refinement, a fibrin film method, has been used to localise such activity to mucosal and submucosal blood vessels).4 More recently a semiquantitative method has been developed in which histological sections of gastroscopic biopsies were placed on a fibrin film; fibrinolytic activity was reflected by the extent of a clear zone.5 We used a sensitive quantitative spectrophotometric assay to measure tissue type and urokinase type plasminogen activator activity and plasminogen activator inhibitor type–1 antigen, in gastric and duodenal mucosal biopsy homogenates. We found that in peptic ulcer disease fibrinolysis is indeed impaired suggesting that vascular damage may play a part in ulcerogenesis.

Patients and methods

PATIENTS
The following groups of patients (total n=62) were studied: non-ulcer dyspepsia – that is, no evidence of current or past ulcer disease or oesophagitis (these served as controls) n=14; active benign gastric ulcer (in the distal body of the stomach or in the antrum but excluding pyloric ulcer) n=15; active duodenal ulcer n=21; healed duodenal ulcer n=12. None of the control patients was receiving histamine H2 receptor antagonists whereas 21 of the other 48 were receiving such treatment.

BIOPSY SPECIMENS
These were taken, wherever possible, from the anterosuperior and posteriorinferior halves of the duodenum, and the lesser and greater curves of the antrum and body. In ulcer patients biopsy specimens were taken from the ulcer edge (or adjacent to the scar in patients with healed

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disease) and from the opposite wall. The biopsy samples were immediately frozen in dry ice and stored at −70°C. The specimens were weighed while deep frozen and those weighing more than 5 mg were processed. The biopsy specimens were then thawed in batches, washed in ice cold 0.9% saline, and homogenised using a Potter Elvehjem homogeniser in 1:25 ml ice cold TRIS/TWEEN buffer (0.1 M TRIS HCl, 0.1% TWEEN-80, pH 7.5). After centrifugation for 10 minutes at 10000 g at 4°C, the supernatants were stored at −70°C in 200 μl aliquots before assay.

**ASSAYS**

Tissue type and urokinase type plasminogen activator activity was measured in the supernatants using the method of de Jong et al., which is a modification of the method according to Verheijen et al. Briefly, the supernatant was mixed with a solution of fibrinogen fragments and plasminogen, which results in the release of plasmin proportional to the amount of plasminogen activators present. The presence of plasmin was detected by its degradation of S2251, a chromogenic substrate, resulting in the development of a yellow colour, which was measured spectrophotometrically at 405 nm. The contribution of tissue type and urokinase type plasminogen activator activity to the release of plasmin was assessed by using specific antibodies against these substances in a four well system (in duplicate), one well without antibodies, the second with anti-tissue type plasminogen activator the third with anti-urokinase type plasminogen activator and the last with both antibodies. Standards for tissue type plasminogen activator and urokinase type plasminogen activator (obtained from Organon Teknika, Cambridge, UK and National Institute for Biological Standards and Control, London, UK respectively) were used in each assay. Plasminogen activator inhibitor type-1 antigen was determined by an enzyme linked immuno-sorbent assay (ELISA) method using the Biopool TintElize kit (Porton, Cambridge, Newmarket, UK). The protein content of the supernatants was measured by a modification of the Coomassie brilliant blue method. Values of tissue type and urokinase type plasminogen activator activity were expressed as milli international units per mg of protein and plasminogen activator inhibitor type-1 as ng per mg protein. The intra-assay and inter-assay coefficient of variation for tissue type plasminogen activator activity were 8% and 15% respectively and for urokinase type plasminogen activator activity 15% and 30% respectively. Inter-assay variation for the protein assay was 7%. Where results were pooled, the mean of the results from the areas being pooled was calculated for each patient (except where a single area was biopsied, when the result from that area was used).

The tissue type and urokinase type plasminogen activator activity values in control samples were distributed in a parametric pattern. Student's t test was therefore used when comparing patient populations. As plasminogen activator inhibitor type-1 was generally undetectable in control samples, a parametric distribution was not shown and the Wilcoxon rank sum method was used. Correlation between tissue type plasminogen activator activity and plasminogen activator inhibitor type-1 was determined by calculating the correlation coefficient and using the t test to determine significance. Probability of less than 0.05 was considered significant.

The study was approved by the Ethics Committee of Rotherham District General Hospital, where all endoscopies were performed, and informed consent was obtained from all patients.

**Results**

**CONTROL PATIENTS**

Distribution of tissue type plasminogen activator activity and urokinase type plasminogen activator activity (Table I)

The mean values of tissue type plasminogen activator in mucosa from the anterosuperior wall of the duodenum were similar to those in mucosa from the opposite wall. Gastric mucosa contained significantly less tissue type plasminogen activator than duodenal mucosa, but its distribution in the lesser and greater curve of the antrum and body was broadly similar. Values of urokinase type plasminogen activator were much lower than those of tissue type plasminogen activator and were marginally higher in the antrum than in the body or duodenum. As there was no significant geographical variation in the distribution of tissue type plasminogen or urokinase type plasminogen activator within the duodenum and within the stomach, for simplicity the results from each organ were pooled in each patient (Table II).

Mean values of tissue type plasminogen activator activity, urokinase type plasminogen activator activity, and plasminogen activator inhibitor type-I (Tables II and III)

The mean values of tissue type plasminogen activator activity in duodenal mucosa (4110 mIU/mg protein) were significantly higher than in gastric mucosa (2800). Values of urokinase type plasminogen activator activity were very much lower, but did not show such variation between duodenum and stomach (150 and 180 respectively). Plasminogen activator inhibitor type-1 antigen was studied in fewer subjects; therefore results from each organ rather than

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Control subjects: geographical distribution of tPA and uPA values expressed as mIU/mg protein (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>Anterosuperior</td>
</tr>
<tr>
<td>No of patients</td>
<td>13</td>
</tr>
<tr>
<td>tPA values</td>
<td>4070 (380)</td>
</tr>
<tr>
<td>uPA values</td>
<td>150 (20)</td>
</tr>
<tr>
<td>Antrum</td>
<td>Lesser curve</td>
</tr>
<tr>
<td>No of patients</td>
<td>13</td>
</tr>
<tr>
<td>tPA values</td>
<td>2970 (300)</td>
</tr>
<tr>
<td>uPA values</td>
<td>170 (20)</td>
</tr>
<tr>
<td>Body</td>
<td>Lesser curve</td>
</tr>
<tr>
<td>No of patients</td>
<td>6</td>
</tr>
<tr>
<td>tPA values</td>
<td>2500 (550)</td>
</tr>
<tr>
<td>uPA values</td>
<td>120 (30)</td>
</tr>
</tbody>
</table>

tPA = tissue type plasminogen activator activity; uPA = urokinase type plasminogen activator activity.
TABLE II  
Fibrinolytic activity of gastroduodenal mucosa in control patients and in patients with peptic ulcer disease. Results expressed as mIU/mg protein, mean (SEM)

<table>
<thead>
<tr>
<th></th>
<th>tPA activity</th>
<th>uPA activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=14)</td>
<td>tPA activity</td>
<td>uPA activity</td>
</tr>
<tr>
<td>Duodenum* (n=14)</td>
<td>4110 (310)</td>
<td>150 (20)</td>
</tr>
<tr>
<td>Stomach* (n=14)</td>
<td>2800 (200)†</td>
<td>180 (20)</td>
</tr>
<tr>
<td>Active duodenal ulcer (n=21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcer edge (n=21)</td>
<td>2220 (350)‡</td>
<td>290 (40)‡</td>
</tr>
<tr>
<td>Opposite wall (n=17)</td>
<td>3460 (370)</td>
<td>200 (30)</td>
</tr>
<tr>
<td>Stomach* (n=17)</td>
<td>2460 (240)</td>
<td>240 (30)</td>
</tr>
<tr>
<td>Healed duodenal ulcer (n=12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scar (n=11)</td>
<td>3290 (310)</td>
<td>310 (50)†</td>
</tr>
<tr>
<td>Opposite wall (n=12)</td>
<td>3750 (480)</td>
<td>190 (20)</td>
</tr>
<tr>
<td>Stomach* (n=12)</td>
<td>3070 (250)</td>
<td>250 (40)</td>
</tr>
<tr>
<td>Active benign gastric ulcer (n=15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcer edge (n=15)</td>
<td>1160 (210)§</td>
<td>210 (30)</td>
</tr>
<tr>
<td>Opposite wall (n=15)</td>
<td>2450 (380)</td>
<td>200 (30)</td>
</tr>
<tr>
<td>Duodenum (n=5)</td>
<td>4830 (510)</td>
<td>190 (30)</td>
</tr>
</tbody>
</table>

* Mean of the results from body and antrum pooled in each patient, as described in methods section. Statistics (t test): compared with control duodenum † p<0.01, ‡ p<0.001; compared with control stomach; § p<0.001.

**TABLE III  
Plasminogen activator inhibitor type-1 in gastroduodenal mucosa (ng/mg protein) (SEM)**

<table>
<thead>
<tr>
<th></th>
<th>PAI-1 (ng/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls duodenum (n=6)</td>
<td>&lt;0.2-0 (0)</td>
</tr>
<tr>
<td>Stomach* (n=6)</td>
<td>&lt;0.2 (0-1)</td>
</tr>
<tr>
<td>Duodenal ulcer edge (n=6)</td>
<td>0.8 (0-3)†</td>
</tr>
<tr>
<td>Gastric ulcer edge (n=6)</td>
<td>1.4 (0-6)‡</td>
</tr>
</tbody>
</table>

* Pooled data from body and antrum. Statistics (Wilcoxon rank sum test); † p<0.05 compared with control duodenum; ‡ p<0.05 compared with control stomach.

different parts of it are presented (Table III). The limit of detection was 0-2 ng/mg protein. In control subjects it was undetectable – that is, <0.2 mg/mg protein – in all biopsy specimens. Apart from two, both from the same subject, one from the antrum (0.9 ng/mg protein) the other from the body (0.3 ng/mg protein).

ULCER PATIENTS

Duodenal ulcer
At the edge of active duodenal ulcers mean tissue type plasminogen activator activity values were significantly reduced (2220) compared with those in control duodenal mucosa; there was also a moderate reduction in the opposite wall, which looked normal on endoscopy examination (Table II). In patients with healed ulcer tissue type plasminogen activator activity values at the scar were low but not significantly so; there was a slight reduction in the opposite wall.

By contrast with tissue type plasminogen activator, the mean urokinase type plasminogen activator activity value at the ulcer edge was raised significantly (290) compared with control values; there was also some increase in the opposite wall. After healing the values were still significantly raised at the scar and slightly raised at the opposite wall. The values of plasminogen activator inhibitor type-1 at the ulcer edge were raised in five of six patients studied, and undetectable in the other; the mean value (0.8) was significantly higher than in controls (Table III).

Benign gastric ulcer
At the edge of active gastric ulcers tissue type plasminogen activator activity values were significantly reduced (1160) compared with those in control gastric mucosa and marginally reduced at the opposite wall, which at endoscopy looked normal (Table II). By contrast the urokinase type plasminogen activator activity values were slightly raised at the ulcer edge and opposite wall. Plasminogen activator inhibitor type-1 values were raised in five of six patients and undetectable in the last; the mean value (1.4) was significantly raised (Table III). Patients with healed gastric ulcer were not studied.

Relation between tissue type plasminogen activator activity and plasminogen activator inhibitor type-1 (PAI-1) at the edge of peptic ulcers.

Effect of H2 receptor antagonists on fibrinolysis
Of the patients with active ulcer disease, 10 of 21 duodenal ulcer patients and 6 of 15 gastric ulcer patients were receiving H2 receptor antagonist treatment at the time the biopsy specimens were obtained; five of 12 patients with healed duodenal ulcer were receiving treatment. There was no consistent change in tissue type plasminogen activator or urokinase type plasminogen activity values associated with H2 receptor antagonist treatment (data not shown).

Discussion
We have found that in both duodenal and gastric ulcer disease there is an appreciable reduction of tissue type plasminogen activator activity in the mucosa at the ulcer edge compared with control mucosa. This cannot be explained simply as a consequence of tissue necrosis for urokinase type plasminogen activator activity showed a simultaneous increase, especially in duodenal ulcer disease; also, in the patients studied plasminogen activator inhibitor type-1 antigen values were increased at the ulcer edge compared with control mucosa. In duodenal ulcers that seemed to have healed when examined by endoscopy (patients with healed gastric ulcers were not
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studied) there was a partial reversion of tissue type plasminogen activator activity towards the normal pattern, but urokinase type plasminogen activator activity remained high. This persistent abnormal fibrinolytic pattern near the scar suggests that this area is not normal and may be vulnerable to further damage. This corresponds to the common clinical finding that re-ulceration often occurs at, or near, the original site. Our findings therefore suggest that the fibrinolytic system participates in peptic ulcer disease.

There have been two previous studies of mucosal fibrinolytic activity in peptic ulcer disease, both using gastroscopic biopsy specimens. In one, in which a fibrin film technique was used, low overall fibrinolytic activity was noted near to ulcers, similar to our findings. The other study, which used an activity assay somewhat different to ours, showed increased tissue type plasminogen activator activity values at the site of gastric ulcers.

Piascicki and colleagues have elegantly shown that the mucosa in the lesser curve of the stomach and in the proximal duodenum is supplied by end arteries; these are the very areas where peptic ulcers occur. This finding takes in conjunction with our findings of reduced fibrinolytic activity at the peptic ulcer edge provides a possible explanation for one of the mechanisms of peptic ulcer formation and its focal nature.

In those hereditarily predisposed to ulcer disease, Helicobacter pylori infection (which is now recognised as being almost universally linked to duodenal ulcer, probably causally, and slightly less often to gastric ulcer) results in intense mucosal inflammation. This leads to release of powerful peptides (such as interleukins) and free radicals, and causes vascular damage that releases other powerful chemicals (for example endothelin-1), which impair the mucosal microcirculation. The reduced blood flow presumably leads to a tendency to thrombosis and if fibrinolytic response is suboptimal mucosal ischaemia may result, especially in the areas supplied by end arteries. If the ischaemia then further damages the ischaemic mucosa resulting in peptic ulcer, the size of which is limited by the vascular sufficiency of the surrounding mucosa. Repair requires the development of new granulation tissue, which needs an efficient fibrinolytic system to maintain blood flow and urokinase type plasminogen activator for tissue remodelling.

Support for the role of the microcirculation in ulcerogenesis is provided by the experimental animal work of Sato et al. They showed that decreased mucosal blood flow and consequent reduced tissue oxygen saturation in the tissue produced changes in the mucosa, which was then damaged when acid was present. The same group subsequently showed, that in humans, using reflectance spectrophotometry through an endoscope, haemoperfusion was reduced at the edge of active ulcers, whereas it was increased during healing vascularity.

Some of our other findings require comment. We found tissue type plasminogen activator activity in duodenal mucosa to be greater than in gastric mucosa, similar to findings in a recent study. Presumably this is of biological importance and may perhaps be linked with the less efficient mucosal blood supply in this area.

We found that plasminogen activator inhibitor type-I almost entirely raised at the ulcer edge especially where tissue type plasminogen activator activity was low. As plasminogen activator inhibitor type-I inhibits the action of tissue type plasminogen activator it may play some part in impairing fibrinolysis. In vitro experiments have suggested that cimetidine acts as an antifibrinolytic agent; indeed Helgerstrand found reduced mucosal fibrinolytic activity in patients treated with H2 receptor antagonists. But our results suggest these drugs have no fibrinolytic effect.

Studies on the pathophysiology of ulcers disease, in particular duodenal ulcer, have been dominated by investigations on acid secretion and its regulation and recently by H pylori. In comparison few investigations have been carried out on mucosal defence. Recently such studies have shown abnormalities, principally impaired duodenal mucosal bicarbonate production and impaired prostaglandin E2 generation at and away from the duodenal ulcer, which persists even after healing. Two other abnormalities can be added: an abnormal mucosal vascular supply and from our data, impaired mucosal vascular fibrinolytic activity.

In conclusion impaired fibrinolytic activity has been found at the edge of peptic ulcers, which may have a bearing on ulcerogenesis.

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