Demonstration of a pH gradient across the mucus layer on the surface of human gastric mucosa in vitro

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SUMMARY In previous studies we have demonstrated a hydrogen ion concentration gradient across the mucus on rat and rabbit fundic mucosa, in vivo and in vitro respectively, observations which support the possibility of a 'mucus-bicarbonate' protective barrier. In the present studies we have demonstrated a similar gradient across the mucus on human gastric mucosa in vitro. The minimum mean hydrogen ion concentration at the mucus-epithelium interface was 1·1×10⁻⁴ mM (pH 6·96, n=10) when the mean luminal concentration was 5·6 mM (pH 2·25). Aspirin (10 mM) and N-acetyl cysteine (306 mM) (5%) increased the minimum intra-mucus hydrogen ion concentration and the gradient was overwhelmed by a luminal hydrogen ion concentration of 40 mM (pH 1·4). These results suggest that a hydrogen ion concentration gradient exists across the mucus on human gastric mucosa and that potential damaging agents may act by compromising one or other of the components of this 'mucus-alkaline', presumed 'mucus-bicarbonate', barrier.

The mechanisms by which the stomach protects itself from potentially damaging acid-peptic secretions are uncertain. Heatley¹ postulated that mucus on the surface of the mucosa provided an unstimulated layer in which bicarbonate secreted by the surface epithelium neutralised hydrogen ions diffusing towards the epithelium from the lumen. Support for this hypothesis has come with the demonstration that fundic mucosa is capable of secreting bicarbonate by an active, energy-dependent, process² ³ and that mucus delays the transfer of small ions across it.⁴ Furthermore, our recent demonstration of a pH gradient across the mucus adherent to rabbit fundic mucosa in vitro⁵ and rat fundic mucosa in vivo⁶ provides direct evidence in favour of such a mechanism. The present studies were designed to examine the possibility that a similar gradient exists across human gastric mucus.

Methods

Gastric mucosa was obtained from gastrectomy specimens taken from 10 patients who had had premedication with pethidine, atropine, or scopolamine, and anaesthetised with thiopentone, nitrous oxide, and halothane together with tubocurarine. The diagnosis was cancer of the stomach in six, gastric ulcer in three, and duodenal ulcer in one. Full thickness specimens were taken from the body of the stomach at least 3 cm away from the margin of any lesion, and placed in oxygenated bicarbonate-free, Ringer's lactate solution. The tissue was mounted horizontally in specially prepared chambers,⁵ within 30 minutes of resection, so that approximately 0·5 cm² of the luminal surface was bathed with 0·5 ml hydrochloric acid (HCl) 10 mM (pH 2·0) and the serosal surface with oxygenated bicarbonate-free Ringer's lactate solution maintained at 37°C. The hydrogen ion activity gradient across the mucus was measured using antimony microelectrodes manufactured as previously described.³ Microelectrodes had tip diameters of approximately 50 μm and these were slowly lowered into and through the mucus using a micro-manipulator which moved the electrode in 42·3 μ steps. Changes in hydrogen ion concentration were recorded in millivolts on a Servoscribe chart recorder via a Vibron voltmeter and converted to hydrogen ion concentrations using calibration curves obtained from standards covering the range 100 mM to 10⁻³ mM (pH 1 to 8). Calibration was carried out each day before each experiment. There was no significant change between the calibration curves before or after insertion into the mucus.

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The possibility that mucus adherent to the epithelial layer of the stomach could have a protective role against intraluminal acid has not received wide support. It has been difficult to envisage mucus resisting the diffusion of small ions through it. Nevertheless, it has been demonstrated that the diffusion of hydrogen ions through a layer of pig gastric mucus occurs four times more slowly than through an equivalent thickness of unstirred saline. Secondly, it is known that fundic mucus can secrete bicarbonate, and that bicarbonate is present within mucus secretions. This combination of an unstirred layer with mucosal alkaline secretion
pH gradient across mucus layer on surface of human gastric mucosa

The observation that 306 mM N-acetyl cysteine, a mucolytic agent, altered the gradient provides support for the importance of the mucus structure in the maintenance of this gradient. Indeed, it has been shown that the gastric mucus layer is reduced in thickness after treatment with NAC. The reduction in the gradient produced by 10 mM aspirin may be relevant to the known damaging effect of aspirin on the gastric mucosa. Aspirin has been demonstrated to have a number of actions any of which could affect a postulated 'mucus-bicarbonate' barrier. The precise mechanism by which it acted here is not known, but the effect could be due to inhibition of alkaline secretion, shedding of adherent mucus, exfoliation of epithelial cells, and/or decreased synthesis of mucus.

Our finding that an increased concentration of HCl will also impair the maintenance of the gradient is of interest. The human stomach can certainly secrete acid of 40 mM or more. However, under physiological conditions this concentration of acid is rarely maintained within the gastric lumen for any length of time. Such stimulated acid secretion would normally be buffered in part by ingested food and evacuated from the stomach.

The results demonstrated here confirm our previous observations that a hydrogen ion concentration gradient exists across the mucus on gastric mucosa and show that this gradient exists in human stomach too. The results with N-acetyl cysteine, aspirin, and HCl 40 mM solutions in human mucosa also confirm observations we initially made in the rat stomach in vivo. The finding that agents which damage the gastric mucosa, such as aspirin, can also affect the gradient, lends support to the proposal that a 'mucus-alkaline' barrier exists on the gastric mucosa. Although we have no direct evidence that human gastric mucosa secretes bicarbonate in vivo, by extrapolation from a number of other mammalian species in which this has been demonstrated, it seems highly likely that this 'barrier' is made up of mucus and bicarbonate.

Table Minimum hydrogen ion concentration (mM), measurable in adherent mucus of untreated stomachs (mean luminal H⁺ concentration, 5-6 mM; pH 2-25) and after 35 minutes' treatment with NAC, aspirin, or HCl (40 mM; pH 1-4)

<table>
<thead>
<tr>
<th>Untreated 'control' mucus</th>
<th>NAC 306 mM n=8</th>
<th>Aspirin 10 mM n=8</th>
<th>HCl 40 mM n=5</th>
</tr>
</thead>
<tbody>
<tr>
<td>[H⁺] mM mean range</td>
<td>2×10⁻²</td>
<td>1×10⁻³-0.5</td>
<td>6×10⁻³-0.5</td>
</tr>
<tr>
<td>pH mean range</td>
<td>4.71</td>
<td>3.01-6.2</td>
<td>4.27</td>
</tr>
</tbody>
</table>

p values are for differences between the untreated and each treated group. There was no significant difference between the three treatment groups.
References


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