Studies of the ‘mucus-bicarbonate’ barrier on rat fundic mucosa: the effects of luminal pH and a stable prostaglandin analogue

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SUMMARY Gastric mucosa may protect itself from acid peptic digestion by maintaining an alkaline barrier zone within the layer of mucus coating its surface. We have measured the pH gradient in the mucous layer in vivo, on the gastric mucosa of anaesthetised rats using antimony chloride micro pH electrodes. The maximum pH recordable adjacent to the epithelium was 7.43±0.56 (n=8) when the luminal bathing solution pH was 2. Adjusting the luminal pH to 7.0 caused the maximal pH to rise to 7.88 (range 7.59 to 8.08), a value which is significantly higher than either luminal or reported intraepithelial pH and suggests that active secretion of alkali is involved. Pretreatment with 16-16-dimethyl prostaglandin E₂ (20 µg subcutaneously) significantly increased the maximal intramucus pH to 7.89±0.45 (n=8) when luminal pH was 2 and prevented the fall in intramucus pH induced by luminal aspirin (20 mM). It did not prevent falls in pH provoked by the mucolytic agent N-acetyl cysteine or by a high luminal activity (pH 1-4). These data indicate that an alkaline environment is maintained adjacent to gastric mucosa and that while this is enhanced by prostaglandin it may be compromised by high luminal acid concentrations or by removal of the support provided by mucus. These observations may be relevant to the mechanisms of gastric mucosal protection against acid peptic damage.

Recent experimental evidence suggests that the gastric mucosa may protect itself against acid peptic digestion by maintaining an alkaline zone in the mucus layer coating its surface. The demonstration, using micro pH electrodes, of a neutral or alkaline pH adjacent to the epithelium when the luminal contents are acid, supports this concept of a ‘mucus-bicarbonate’ barrier. The maximal pH which the gastric mucosa is capable of producing at its surface, however, is uncertain. We have now examined this aspect by measuring the maximal pH in the mucous gel under the most favourable basal conditions and after stimulation with a synthetic prostaglandin analogue 16-16-dimethyl prostaglandin E₂. As this prostaglandin has been shown to prevent a variety of types of gastric mucosal damage we have also examined its ability to prevent the fall, produced by a number of damaging agents, of the pH gradient across the mucous layer.

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Methods

Measurements of the intramucus pH were made as previously described. Briefly, Sprague-Dawley rats fasted for 24 hours were anaesthetised with chloral hydrate and, after the stomach was exteriorised, a pedicle of fundic stomach was prepared. A cuff of polyvinyl tubing was laid on the mucosal surface to form a luminal chamber 0.5 ml in volume, while mucosal blood flow was preserved. This luminal chamber was filled with pH 2.0 HCl at 37°C. The pH gradient within the mucous layer was measured with an antimony microelectrode, tip diameter approximately 100 µm and prepared according to the method of Califsh, Pucacco, and Carter. An indifferent electrode of saturated KCl in 3% agar was positioned in the luminal chamber and changes in potential difference were measured using a Vibron electrometer (Electronic Instruments Ltd, Richmond, UK) and recorded on a Servoscribe recorder (Smiths Industries, London, UK). Antimony electrodes were calibrated before each study using pH solutions 1 to 8, and these gave a
linear response within this pH range. The micro-
electrode was moved vertically through the mucus
layer in 42.3 μm steps using a micromanipulator and
the maximum pH obtainable within the mucus was
recorded.

The effect of 16-16-dimethyl prostaglandin E₂
(16-16-DMPGE₂) 20 μg subcutaneously given one
hour before laparotomy was studied in one group of
rats. The effect on the intramucous pH of changing
the pH of the luminal solution from 2 to 3, 5, or 7
was also determined over a 30 minute period. The
osmolality of these solutions was adjusted 300
mOsm/kg by addition of relevant amounts of NaCl.
The effects of topical treatment with 20 mM aspirin
in pH 2 HCl, 310 mM n-acetyl-L-cysteine (NAC) or
pH 1-4 HCl on the pH measured when the micro-
electrode was held at a constant site within the
mucous layer was determined in control and paired
16-16-DMPGE₂ treated rats over a 15 minute
period. Statistical comparisons were made using
paired t tests.

Results

The maximal pH recorded in the mucous gel in
control rats was 7.43±0.56 when the luminal pH was
2.0 (n=8). It is likely that this maximal value occurs
at the mucus-mucosa interface as further
progression of the electrode tip, in some experi-
ments, was associated with a sudden fall of about 0.5
pH units as the tip entered the tissue.

When the pH of the luminal solution was changed
to pH 3 or pH 5 the pH, measured continuously by a
microelectrode held at a fixed point within the
mucus, tended to rise over the 30 minutes of the
study (Fig. 1). Thus with a luminal pH of 3, intramucous pH rose from 5.6±0.66 to 6.55±0.59
(n=6; p<0.001). With a luminal pH of 5, intramucous pH rose from 5.25±0.5 to 6.11±0.47 (n=6;
p<0.001). It is clear then that the luminal pH
influences intramucous pH.

In order to determine the maximum pH which the
mucosa was capable of producing, the pH of the
luminal bathing medium was adjusted to 7
(phosphate buffer). In five rats the maximum pH
recorded was 7.88±0.2 (range 7.59±0.08) and this
was maintained for the 30 minutes of the study. At
the end of this period the luminal bathing medium
pH was 6.85±0.33.

In the animals given 16-16-DMPGE₂ the
maximum intramucous pH was 7.89±0.45 (luminal
pH 2) compared with that in the paired control
animals of 7.43±0.56 (n=8; p<0.05).

After addition of aspirin (20 mM) to the luminal
solution, the intramucous pH fell in control animals
while this fall was prevented by pretreatment with
16-16-DMPGE₂ in paired rats (Fig. 2). The mean
pH values for these two groups of rats were
6.78±0.73 and 5.30±1.42 at 0 and 15 minutes after
aspirin in control animals (n=8; p<0.05) and
6.88±1.02 and 6.85±0.84 in the 16-16-DMPGE₂
pretreated animals (n=8; NS) (Fig. 2).

Addition of NAC to the luminal solution caused a
fall in constant pH values in rats pretreated with
16-16-DMPGE₂, from 6.47±0.77 to 4.99±1.60 at 15
minutes (p<0.02) and in untreated animals from
5.69±1.13 to 3.88±0.61 (p<0.001). There was no
significant difference between the response in 16-16-
DMPGE₂ pretreated and control rats.

Topical application of pH 1-4 HCl caused a fall in
constant pH in rats pretreated with 16-16-DMPGE₂,
from 7.24±0.70 to 5.73±1.37 (n=8; p<0.01) and in
control rats from 6.86±1.12 to 5.50±1.51 (n=8;
p<0.01). There was no difference between treated
and control rats.
Discussion

We have recently shown the existence of a gradient of pH across the layer of mucous gel on the gastric mucosa of rat, rabbit, and man. These observations have recently been confirmed in the frog. The pH adjacent to the mucosa was near neutral when luminal pH was acid supporting the concept of a 'mucus-bicarbonate' barrier. Secretion of the alkaline component of this presumed protective barrier has been studied in some detail and has been shown to be an active process. A number of prostaglandins have been shown to stimulate gastric alkali secretion and to prevent the fall in secretion provoked by non-steroidal analgesics. It is possible therefore that endogenous prostaglandins have a role in maintaining the alkaline juxta mucosal zone. It was of interest therefore to examine the maximal pH recordable in the mucous layer under basal conditions and after stimulation with prostaglandin. This appears to be about 7.88 and is clearly alkaline when luminal pH is neutral. The prostaglandins too increased intramucous pH to this level even when the luminal bathing fluid was acid (pH 2). These values are greater than interstitial fluid pH and almost certainly higher than intracellular pH and thus provide further evidence for an active process for alkali (presumably bicarbonate) secretion.

We have previously shown that topical aspirin disrupts the mucus pH gradient in rat in vivo and in human, in vitro. This effect has also been noted in the intramucus pH gradient which exists on rat duodenal mucosa. Damage by aspirin could be caused by shedding of adherent mucus, exfoliation of surface epithelial cells, decreased synthesis of mucus, or by inhibition of bicarbonate secretion. Prostaglandins are protective against aspirin and acid induced ulceration, even at doses insufficient to inhibit acid secretion. In our experiment the disruptive effect of aspirin was nullified by prior treatment with 16-16-DMPGE2, supporting the suggestion that it probably acts by preventing aspirin induced inhibition of bicarbonate secretion and possibly by increasing the mucus layer thickness. The damaging effect of a highly acid luminal pH (1-4), however, was not abrogated by 16-16-DMPGE2. Presumably, even in 16-16-DMPGE2 stimulated stomach, there is insufficient alkali output to neutralise a constant excess of luminal pH 1-4 HCl.

These observations indicate that the 'mucus-bicarbonate' barrier has a limited capacity to prevent acid reaching the mucosa. A luminal pH of 1-4 or less is not uncommonly reached for varying periods of time after meals and during fasting suggesting that disposal of the excess acid which reaches the mucosa must be achieved by some alternative mechanism. Viewed in this light the 'mucus-bicarbonate' barrier might be considered as a first line defence, and second line defence mechanisms, perhaps dependent on adequate mucosal blood flow, must be of some importance.

Reduction of the pH gradient by NAC, was again unaffected by prior 16-16-DMPGE2 treatment. Measurements of mucous gel thickness have shown that NAC thinned the mucous layer to a similar degree in prostaglandin treated and non-treated mucosa thereby negating the protective effect of

Fig. 2 Effect of aspirin (20 mM) in luminal fluid on intramucus pH in rat fundic mucosa. In untreated rats (right hand panel) there was significant fall in mean pH (p<0.05) but this fall was prevented by pretreatment with 16-16-DMPGE2 (left hand panel).
mucus. These data emphasise the importance of mucus for the support of the alkaline buffer zone.

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References

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