MUCUS AND H. PYLORI

A continuous, adherent mucus gel layer with mucosal bicarbonate secretion is the initial protective barrier in the stomach and duodenum against erosion by the gastric juice. *H. pylori* resides within the adherent mucus gel layer close to the epithelial surface.

The barrier function of the mucus layer in vivo depends on (i) its thickness, and (ii) its gel structure, a property which is linearly dependent on the polymeric mucin content. We have shown in vivo that *H. pylori* colonisation alone did not decrease the thickness of the adherent gastric mucus barrier, although there was a mean 20% decrease in mucus thickness in those *H. pylori* positive subjects with underlying gastric atrophy. There was, however, a significant mean 18% reduction in the gel-forming polymeric mucin content of mucus from *H. pylori* subjects, independent of underlying atrophy. Studies in vitro suggest this loss of gel structure might arise from a *H. pylori* mediated, high local pH generated by urease activity rather than by proteolysis.

This study shows that *H. pylori* infection alone does not compromise the overall integrity of the mucus barrier in vivo. However, in the immediate environment of the organism there appears to be a localised loss of mucus gel structure. The mucus barrier is compromised if *H. pylori* associated gastric atrophy or peptic ulceration follows.

**Key words:** mucus, pepsin, bicarbonate, peptic ulceration

**INTRODUCTION**

The gastric mucus barrier exists as a layer of mucin gel adherent to the surface of the mucosa and provides a protective interface between the secreting epithelium and the gastric juices in the lumen (*Fig. 1*) (1, 2). In man the gastric mucus gel layer is continuous and of variable thickness with values between 30 µm—310 µm and 95% of the readings between 50 µm—200 µm (3, 4).

The gastroduodenal mucus bicarbonate barrier against acid is well documented both in man and experimental animals (1, 5, 6). Epithelial

bicarbonate secretion neutralises luminal acid within the matrix of the adherent mucus gel. A pH gradient is established from an acid pH1-2 in the lumen to a near neutral pH7 at the mucus-epithelial surface. Such pH gradients have been demonstrated using pH sensitive microelectrodes in vivo in man and a variety of experimental animals (1, 7). The primary function of the mucus gel is to create a stable, unstirred layer at the mucosal surface which acts as a mixing barrier, preventing the newly secreted $\text{HCO}_3^-$ from being overwhelmed by the vast excess of acid in the lumen. The mucus bicarbonate barrier is considered the first line of defence against acid, particularly in the duodenum. In the stomach, luminal pH values as low as pH1 are attained, and under these circumstances experimental evidence suggests that pH gradients collapse resulting in values as low as pH1 occurring next to the mucosal surface. In such situations, other mechanisms of mucosal defence at the level of the rapidly repairing epithelial cell layer and leakage of the $\text{HCO}_3^-$ rich interstitial fluid come into action (1).

The mucus layer is permeable to small molecular weight solutes and ions but acts as a diffusion barrier to large molecular weight pepsins ($\text{Mr} \sim 30\text{ K}$) over a physiological timescale (1, 8). Therefore, the continuous layer of adherent mucus gel over the gastric mucosa will prevent pepsin in the lumen from gaining access to the proteolytic sensitive underlying epithelia. Luminal pepsin will digest the surface of the mucus gel to produce soluble degraded mucin in the gastric juice (8, 9). The continuity of the mucus gel layer seen in vivo is evidence that, normally, mucus secretion balances that lost by peptic digestion and mechanical erosion. Instillation of pepsin, in excess of maximal secretion, into the rat stomach in vivo will overwhelm the mucus barrier with a large increase in soluble, degraded mucin, and small focal haemorrhagic lesions with bleeding into the lumen (8). Discontinuities are seen in the adherent mucus layer and localised punctate ulcers appear due to peptic

Fig. 1. A diagrammatic representation of the mucus: bicarbonate barrier and the location of $H.\text{ pylori}$ at the base of the mucus gel.
digestion of the epithelium. Such experiments provide evidence of a key role for the mucus layer in gastroduodenal mucosal protection.

*H. pylori* is concentrated within the mucus gel layer at its base close to the mucosal surface, particularly in the location of the gastric pits (*Fig. 1*) (10, 11). The matrix of the mucus gel layer creates a stable unstirred aqueous microenvironment for the organism. The spiral shape of *H. pylori* and its flagellae enable the organism to penetrate and colonise the mucus gel layer (12). *H. pylori* is sensitive to levels of acid normally present in the gastric juice, pH3 or less (13). Once established within the mucus layer, the *H. pylori* organisms will themselves be protected from the low pH of the gastric juice by the pH gradient of the mucus:bicarbonate barrier (14). A further line of defence against low pH for *H. pylori* is local generation of ammonia mediated by its secretion of urease (12, 15).

*H. pylori* infection results in chronic gastritis and can lead to the development of peptic ulceration (10, 16). Our previous work on mucosal resections has shown that the structure of the mucus barrier is impaired in peptic ulcer disease (17). Further it has been proposed from *in vitro* studies that *H. pylori* compromises mucus barrier function (18, 19), however, this is controversial (20—22). Here we describe the effect of *H. pylori* infection on two key structure/functional parameters of the gastric mucus barrier, the thickness of the mucus layer and the gel-forming polymeric structure of the component mucins. Our studies have applied new micro-methodology for investigating *in vivo*, mucus thickness and mucin polymeric structure using endoscopically obtained biopsies and mucosal brushings.

**THE EFFECT OF H. PYLORI ON THE THICKNESS OF THE ADHERENT MUCUS LAYER IN VIVO**

The maintenance of a continuous mucus layer with a minimum effective thickness to fully support the pH gradient and resist erosion by pepsin mechanical shear etc., is essential for its protective functions. Until now, mucus thickness in man has only been successfully measured using unfixed mucosal sections (23). This has been necessary since normal histological procedures for preparing and staining mucosal sections involve prolonged use of organic solvents and mountants which dehydrate, distort and remove the mucus gel layer, from the mucosal surface (24). We have recently developed a histological method which fully preserves the adherent mucus gel layer, minimising the use of organic solvents and employs a water soluble mountant (3). This method can be applied to cryostat sections of endoscopically obtained biopsy samples and in animal models gives mucus thickness values comparable to those seen *in vivo.*
We have investigated mucus thickness in biopsy specimens obtained from 20 H. pylori negative and 20 H. pylori positive subjects (4). H. pylori status was confirmed by a combination of both positive histology and positive serology. All subjects had a macroscopically normal stomach and were not taking either NSAIDS or acid suppressive therapy. Cryostat sections of the biopsies were processed and stained using a modified periodic acid Schiff/Alcian blue stain (PAS/AB) according to the method of Jordan et al. (3).

A thick, continuous PAS positive mucus layer was observed over the surface of gastric mucosal sections from all subjects. H. pylori negative subjects had a mean (SD) mucus thickness of 106 (30) µm. All the H. pylori positive subjects (aged matched, mean 59 years) had a mean (SD) mucus thickness of 94 (24) µm which was not significantly different from that in uninfected subjects (4). When gastric atrophy was considered, 9 H. pylori positive subjects with atrophy had a significant 18% reduction (p = 0.03) in mean (SD) mucus thickness, 84 (13) µm, compared to those subjects with H. pylori infection but without atrophy who had a mean mucus thickness of 104 (26) µm, comparable to H. pylori negative subjects. These results show that H. pylori infection alone does not reduce the thickness of the adherent mucus barrier and thus the cover of a protective stable unstirred surface gel layer is preserved. It is only with H. pylori associated gastric atrophy that there is a significant reduction in mucus thickness, although the mucus layer remained continuous and all individual readings were 30 µm or above with 95% of the readings greater than 50 µm.

An earlier report using the unfixed mucosal section technique for mucus thickness measurements on biopsy specimens, reported up to a 50% reduction in gastric mucus thickness with H. pylori infection (19). However, in this study neither the effects of gastric atrophy or ulcer pathology were considered. Also, the unfixed section technique, usually applied to mucosal resections (23) in our hands is very difficult on biopsies and gives variable results.

**MUCIN POLYMERIC STRUCTURE AND GEL STRENGTH OF THE MUCUS BARRIER**

The effectiveness of the adherent mucus barrier is also dependent on the stability and structural quality of the gel itself. A weakening of the gel structure could make the mucus barrier more easily eroded by pepsin and mechanical shear as well as possibly compromising the unstirred layer properties of the gel matrix. They key structural feature of the gel-forming mucins is their covalent polymerisation mediated by disulphide bridges between mucin subunits (2, 24). Proteolysis breaks that part of the mucin protein core bearing inter-subunit disulphide bonds to produce highly glycosylated mucin fragments that do not form a gel. *In vitro* rheological studies, using mechanical spectroscopy, on
gastric mucus gel have shown a direct correlation between the percentage of polymeric mucin relative to degraded mucin and the measured mechanical gel strength of the mucus preparation, (Fig. 2), (25). Our early work studying resected human gastric mucosa using gel filtration fractionation, showed that the amount of polymeric mucin relative to degraded mucin in adherent mucus was halved in subjects with chronic gastric ulcer (17). Thus, 67% of the mucin was polymeric in resected mucosa from non-ulcer controls, subjects undergoing a Whipples resection for pancreatic cancer. In contrast, only 35% of the mucin was polymeric in adherent mucosa from chronic gastric ulcer patients. Relating this to in vitro rheology studies (Fig. 2), it can be seen that this reduction in polymeric mucin in gastric ulcer subjects signifies a substantial collapse of the mucus gel structure, to the extent that it would be expected to markedly compromise the stability of the mucus barrier.

![Fig. 2. The relationship between gel strength, measured by mechanical spectroscopy, and the percentage of mucin that is in the polymeric form. Rheological data is taken from Sellers et al. 1988 (20); note decreased tan Δ values correspond to increasing gel strength and the transition from gel properties to viscous liquid properties occurs when tan Δ rises to 1. Percentage of polymeric mucin in mucus from resected ulcer patients taken from Younan et al. 1982 (17) and in biopsies from normal and H. pylori subjects from Newton et al. 1996 (4).](image)

The above studies predated the recognition of the key role of *H. pylori* in gastroduodenal pathology and pharmaceutical developments of H₂ blockers have largely eliminated the need for routine resection in chronic peptic ulcer disease. Therefore, a key and current question arising out of the earlier study on the breakdown in polymeric mucin in peptic ulcer disease is the effect of *H. pylori* infection alone. To address this problem, it was necessary to develop a quantitative micro-method for estimating the ratio of polymeric to degraded mucin in the small amounts of mucus obtained from biopsy samples. We have now developed such a method using polyacrylamide gel electrophoresis to
separate the large molecular weight polymeric mucin \((\text{Mr} \geq 2 \times 10^6)\) which is retained in the stacking gel from the smaller degraded mucin \((\text{Mr} \approx 5 \times 10^5)\) which enters the running gel (22). The mucin prior to fractionation by SDS PAGE was first purified by density gradient centrifugation in CsCl. Following electrophoresis, the mucin bands were identified and quantitated by PAS staining by scanning at 555 nm.

To investigate the effect of \(H. \text{ pylori}\) on mucin polymeric structure, endoscopic samples were obtained from age-matched 24 \(H. \text{ pylori}\) negative subjects and 24 \(H. \text{ pylori}\) positive subjects (26). From each individual, two groups of samples were studied: a) pooled mucosal surface brushings obtained by sweeping a cytology brush four times across the antrum, a sample of the adherent mucus gel layer; b) four pooled antral biopsies which contained both intracellular mucin and adherent extracellular gastric mucus gel. The means (SD) ratio of polymer to small sized degraded mucin in antral brushings from \(H. \text{ pylori}\) positive individuals was 59.5(16)\%. This was significantly 18\% lower \((p = 0.01)\) than the mean ratio of 72(12)\% in \(H. \text{ pylori}\) negative individuals. Thus, \(H. \text{ pylori}\) does cause a significant breakdown in polymeric mucin structure \textit{in vivo} within the adherent gel in which it lives. Interestingly, there was only a 5\% reduction, not significant, in the mean (SD)% ratio of polymer to degraded mucin in biopsies from the same \(H. \text{ pylori}\) positive patients, 76(13)% compared to \(H. \text{ pylori}\) negative patients 80.5(9)\%. Since biopsies will also include a substantial amount of stored presecreted mucus, in addition to the adherent mucus, this emphasises that the \(H. \text{ pylori}\) induced collapse in polymeric structure is probably confined to the adherent mucus gel. This would strongly suggest that \(H. \text{ pylori}\) is causing a breakdown in polymeric structure of its own mucus gel environment rather than an effect on mucin biosynthesis.

The \(H. \text{ pylori}\) induced decrease in polymeric mucin content when compared with rheological data from \textit{in vitro} studies (Fig. 2) would indicate that overall the mucus gel structure is still relatively intact. This is supported by the absence of any change in thickness with \(H. \text{ pylori}\) infection as discussed above. A reasonable interpretation of this data is that \(H. \text{ pylori}\) is affecting its immediate environment and causing a local dissolution of the mucus gel while the overall adherent mucus layer remains. One previous study \textit{in vivo} has shown that the viscosity of gastric mucus obtained from gastric washouts was significantly higher in patients infected with \(H. \text{ pylori}\) compared to that after eradication of the infection (27). However, several studies have shown that changes in luminal mucus are not comparable to the adherent gel layer (1, 18). In fact a possible explanation of this higher viscosity of luminal mucus from \(H. \text{ pylori}\) infected subjects might be that larger fragments of intact gel are released by erosion into the gastric juice from the adherent mucus layer due to an inherent weakening of the gel in infected subjects, as shown by our observations.
The overall mechanism by which H. pylori breaks down the mucus gel is uncertain. A H. pylori protease would be an obvious explanation and one study has reported the isolation of such a mucolytic protease from the supernatants of H. pylori cultures in vitro (18). However, subsequently, three different laboratories have published studies failing to confirm the existence of substantial H. pylori protease activity (20—22).

Another possibility for the breakdown of mucus gel in H. pylori infection is that it results from increased pepsin mucolytic activity in the gastric juice. Such increased mucolytic activity in association with changes in pepsin subtypes has been shown in peptic ulcer disease (9). Our preliminary studies measuring 24 hr pepsin activity in H. pylori positive and H. pylori negative patients suggest this unlikely (28). H. pylori positive subjects showed a dramatically different diurnal profile from age matched uninfected subjects with a significantly lower baseline of pepsin activity during the day, although pepsin activity was comparable between both subjects after the evening meal and during the early part of the night.

One plausible explanation that might explain, at least in part, the breakdown of the mucus gel by H. pylori is the relatively high alkaline conditions resulting from its urease activity (21, 22). Transmucosal diffusion of plasma urea occurs and generation of 10 mM NH₄OH at the mucosal surface is possible in vivo since normal plasma urea levels are around 5 mM. We have demonstrated a 23% drop in mucin viscosity on incubation with 10 mM NH₄OH (pH 10.2) and this was accompanied by a decrease in the ratio of polymeric to degraded mucin from 76% to 54%. Further, when an H. pylori cell sonicate was incubated in vitro with excess urea and gastric mucin at 37°C the pH rose to 9.5 and a 23% decrease in mucin viscosity occurred compared to mucin alone or mucin plus urea controls (22). These results show that H. pylori urease could create in vivo a sufficiently high alkaline pH of 9.5—10 to cause some breakdown of mucin polymeric structure. At the base of the mucus gel layer, a near neutral pH will exist much of the time by virtue of the pH gradient. Under these conditions the H. pylori urèase mediated rise in pH would result in the local breakdown of the mucus gel matrix to produce a viscous local environment around the bacterium which would allow it some freedom of movement.

CONCLUSION

The overall conclusion from these studies is that serious breakdown of the mucus barrier is not a direct consequence of the presence of H. pylori alone. In fact, the organism appears to have developed a mechanism which allows motility through its immediate gel environment without comprising the overall
protective properties of the adherent mucus barrier in which it lives. The mucus barrier does not appear to be seriously compromised with *H. pylori* infection unless peptic ulceration occurs.

**REFERENCES**


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