Barrier Function of Gastric Mucus
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Abstract. A viscoelastic mucus gel layer covers the gastric mucosa in a continuous sheet. The functions of the mucus gel have been one of the least studied aspects of gastric barrier function. Although the role of gastric mucus in providing physical protection against ingested particles, and preventing contact between digestive enzymes such as pepsin and the underlying mucosa is generally accepted, the barrier role function of gastric mucus with regard to luminal acid is still conjectural. The modest proton diffusion barrier that mucus provides is negligible in relation to the overall barrier properties of the gastric mucosa; nevertheless, stabilization of unstirred layers and damping of rapid shifts in luminal pH are potentially important functions. Associative studies have suggested a possible role of a hydrophobic barrier in strengthening the barrier functions of mucus. One of the most actively investigated areas of mucus function in recent times has been the mechanism by which secreted acid traverses the gel. Although compelling and complementary data obtained in vivo and in vitro have been consistent with secretion of acid under pressure, creating temporary viscous fingers through the gel, recent evidence obtained with in vivo confocal microscopy suggests that secreted acid diffuses through the gel. Since Helicobacter pylori exists solely in the juxtamucosal portion of the gastric mucus gel, detailed knowledge concerning the pH microenvironment in which the organism thrives is important in understanding the pathophysiology of peptic ulcer disease and related conditions. (Keio J Med 48 (2): 63-68, June 1999)

Key words: stomach, acid, diffusion, injury, bicarbonate

Introduction

The gastric mucosal barrier to acid is a laminar structure, consisting of a pre-epithelial mucus-bicarbonate layer, an epithelial layer, and a post-epithelial layer consisting of blood vessels, non-epithelial cells and enteric nerves. Of these layers, the role of the pre-epithelial mucus-bicarbonate gel is the least well understood. In this review, I will discuss the protective function of the mucus gel in delaying the flux of protons between lumen and the epithelium. Furthermore, the mechanism by which secreted acid traverses the mucus gel en route to the gastric lumen will be addressed. This paper is intended to update the reader on recent developments in the understanding of proton diffusion through gastric mucus; the reader is directed to an excellent general review of gastroduodenal mucosal protection,\(^1\) and to several reviews of the protective role and barrier function of gastric mucus\(^2-5\) to obtain further details.

Proton Back Diffusion

Trans mucus gel permeability

A millionfold proton concentration gradient can exist between the gastric lumen and the blood. The concept of back diffusion embodies the notion that, due to the high concentration gradient, secreted acid diffuses through the gastric mucosa into the blood stream by a process known as back-diffusion. The first barrier encountered by back-diffusing acid is the mucus gel. To understand the role that mucus may play in the overall barrier function of the gastric mucosa, the proton permeability (\(D_{H^+}\)) of mucus has been measured in vitro in Ussing chambers. Most studies suggest that \(D_{H^+}\) for

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mucus is approximately 25% of $D_{H^+}$ in free solution. This modest decrease in permeability can not, of itself, add much to the overall barrier function of the gastric mucosa. $D_{H^+}$ in free solution at 37°C is $6.6 \times 10^{-5}$ cm$^2$/sec. A typical $D_{H^+}$ through native (unprocessed) gastric mucus is 1.75 $\cdot$ $10^{-5}$ cm$^2$/sec. This latter figure translates into a flux through a 100 μm thick mucus gel of ~8 μmol/cm$^2$/sec. Transmucosal proton flux can be roughly estimated by measuring the decrease in proton concentration in a pylorus-ligated stomach in which acid secretion has been maximally inhibited. Assuming a surface area of 10 cm$^2$ for a rat stomach, transmucosal proton flux, calculated in this fashion is only 0.008 μmol/cm$^2$/sec, only 0.1% of the proton flux per area that is crossing the mucus. Thus, the modest permeability barrier presented by gastric mucus in and of itself probably plays little if any role in protecting the underlying epithelium from luminal acid.

**Possible protective function of mucus**

Since mucus is likely to play only a small role in terms of its overall permeability to proton diffusion, what other functions might it have? One of the most logical functions of mucus is to stabilize a transgel pH gradient. Numerous studies have documented, with the use of microelectrodes slowly advanced through the gel, that the pH within the mucus gel gradually increases as the electrode is advanced towards the mucosa. With luminal pH above 2, pH values as high as 7 have been measured juxtamucosally (just above the luminal membranes of the surface epithelial cells). The genesis of this pH gradient is believed to be due to the neutralization of inwardly permeating protons by secreted bicarbonate ions. In order to achieve a stable pH gradient, it is thought that a modest permeability barrier, as provided by the mucus gel, is sufficient to stabilize an unstirred layer, enabling the perpetuation of a pH gradient. An example of a gel-stabilized gradient would be the concentration gradient surrounding the well of an Ouchterlony agar plate. This hypothesis, though logical and appealing, has been questioned, since microelectrode measurements indicate that the pH gradient can extend beyond the surface of visible adherent mucus. Recent observations of gel pH have also provided data inconsistent with this hypothesis (see below).

Another possibility is that the mucus, even with its modest permeability barrier, plays an important role in damping the rapid shifts of proton concentration that occur in the stomach after meals. In humans, 24 hour pHmetry indicates that proton concentrations in the bulk luminal fluid can change 3 log orders in 10 minutes. Such rapid changes in luminal pH would stress the epithelial cells, which have homeostatic mechanisms, such as sodium-proton exchange, that actively remove influxing protons from the intracellular milieu, preserving cell viability. Since some of these mechanisms take several minutes to be maximally activated, the modest permeability barrier presented by gastric mucus could effectively damp the large fluctuations of luminal pH, providing a more gradually changing environment for the epithelial cells. The importance of the mucus gel in preserving intracellular pH ($pH_I$) during luminal acidification has been shown repeatedly in rat gastric mucosa, where perturbations that decrease transgel proton permeability also preserve $pH_I$ during acid challenge (for a review, see 12). For example, treatments such as bismuth salts and human spasmyloytic peptide both help preserve $pH_I$ during acid superfusion in an in vivo preparation of gastric mucosa during acid challenge by increasing the barrier function of mucus, without altering other known defensive mechanisms such as mucosal blood flow. Both treatments decreased the $D_{H^+}$ of mucus without affecting its thickness, thereby enhancing its barrier function.

**The Hydrophobic Layer**

Several groups have documented the existence of a hydrophobic layer at the luminal surface of gastric mucus, although an earlier paradigm suggested that the apical membrane of the epithelial cells itself was hydrophobic. The layer is thought to impede inward proton flux by virtue of its hydrophobic nature. Indeed, correlative studies of mucus hydrophobicity, as measured by contact angle goniometry, and gastric mucosal injury, have demonstrated clear associations between this measure and gastroprotection. For example, prostaglandins, which are known gastroprotective compounds, increase the contact angle (hydrophobicity) of the mucus gel, whereas damaging compounds, such as NSAIDs, decrease the contact angle. Hydrophobicity has also been measured in clinical circumstances, such as *Helicobacter pylori* infections, which decrease the contact angle. Criticisms of this theory, however, have been directed at the technique, which involved drying the mucosa prior to contact angle measurement, and the lack of supportive evidence obtained in vivo that the hydrophobic layer actually impedes proton permeation. The thermodynamic stability of a lipid monolayer, as envisioned by some investigators, has also been questioned. Nevertheless, the concept of a hydrophobic layer is attractive inasmuch as it might decrease $D_{H^+}$ much more than would be measured in Ussing chambers. Furthermore, we have observed that carbon particles, which are hydrophobic, adhere readily to the surface of gastric mucus in vivo, but not to duodenal mucus, suggesting differing surface characteristics of the mucus secreted by either organ.
Role of Mucus in Proton Secretion

Until recently, the path by which secreted acid traversed the mucus gel has never been well understood. In the past, the impedance of the flux of secreted acid by mucus was either simply ignored, or was considered to occur by diffusion.

The role of viscous fingering

In the early 1980’s, the technique of in vivo microscopy was used to visualize the effects of acid secretion at the mucosal surface. One of the most striking findings was accomplished with the use of the dye Congo red, a pH indicating dye that adheres to the luminal surface of the mucus gel. Upon stimulation of acid secretion, a color change was noted in the dye in small areas overlying the openings of the gastric pits, indicating that local acidification had occurred. This finding was interpreted as being consistent with tunneling of secreted acid through gastric mucus through discrete channels. Further support for the tunneling hypothesis was provided several years later, when in vitro experiments confirmed that the general concept of viscous fingering applied to the specific case of acid traversing mucus. Acid injected through a small gauge needle under pressure will tunnel through a gel, which is quite different than its behavior when injected into a liquid, which is free mixture with the liquid. When mucus pH was reduced to <4, tunneling was not observed, presumably due to the increase in mucus viscosity.

On the basis of these two complementary observations, the concept of secreted acid tunneling through the mucus gel by the process of viscous fingering was generally accepted. Further data supporting this hypothesis has been provided largely by Holm’s group in Sweden. With the use of sensitive micro-transducers, fluctuating pressures were measured in the lumen of the gastric glands, consistent with the existence of a gland luminal hydrostatic pressure. Further studies have examined the regulation of gland luminal pressure by acid secretagogues and antisecretory agents and also have demonstrated the existence of muscle fibers surrounding the glands, the contraction of which would presumably generate intraluminal pressure. Pressure, however, can only be generated against a resistance, which is postulated to be generated by constriction of the luminal opening of the gastric pit simultaneously with squeezing the gland. Such a constriction, and direct observation of secreted acid traversing the mucus gel by viscous fingering, however, have not been reported.

Measurement of gel pH with in vivo confocal microscopy

The most recent investigation of mucus pH has been done with the help of a new technique, developed by Drs. Montrose and Chu in collaboration with Dr. S. Tanaka of my laboratory in which confocal microscopy was used to non-invasively measure mucus gel pH in vivo. A ratiometric technique was used to quantitatively report fluorescence response from pH-dependent and pH-independent fluorescent dyes as a measure of pH. Images of pH had spatial resolution of better than 1 μm, and were collected in 1 second. Figure 1 depicts confocal reflectance images of the mucosa parallel (A) and perpendicular (B) to the mucosal surface. Gel pH was then measured with either pH3 or pH5 superfusion (to simulate the fasted and fed states, respectively), and in the presence of secretagogues or proton pump inhibitors. During pH3 superfusion, juxtamucosal pH was near pH 4 and increased to over 5 after PGE2, consistent with prior observations of an alkaline juxtamucosal layer (Fig. 1C). Nevertheless, with pH5 superfusion, juxtamucosal pH was 4.2 (Fig. 1D), and could be acidified further to 3.7 during maximal acid stimulation with pentagastrin. The mucus gel, due to its content of cellular debris, was easily visualized (Fig. 1B) and often was exceeded in thickness by the depth of the acid layer. This acid layer was uniform above the mucosal surface, and the depth of the layer was related to the rate of superfusion. Prior work suggested that viscous fingers of secreted acid should have been observed to be 15 μm in diameter, but no inhomogeneity was observed, even during maximal acid stimulation, and even when observed directly over the mouths of the pits. By contrast there was some inhomogeneity observed during pH3 superfusion, when juxtamucosal pH was high but when there was no evidence of active acid secretion (only base secretion was evident). In this condition, underlying base (presumably bicarbonate) secretion appeared to emanate mostly from the surface cells lining the gastric pits. These data were interpreted as being inconsistent with the viscous tunneling hypothesis for acid secretion. Instead, secreted acid diffused laterally and then towards the lumen. The reason why these experiments differed from prior studies is not yet clear, but was not due to a lack of robust acid secretion or an inability to detect an alkaline surface pH.

Asymmetry of Proton Diffusion

Protons are constantly traversing the gastric mucus in two directions. Secreted acid emanating from the gastric pits crosses the mucus before reaching the lumen, whereas luminal acid must cross the gastric mucus before reaching the epithelial cells and deeper struc-
Fig. 1 Imaging gastric mucosal epithelial surface, mucus gel, and surface pH gradient with in vivo confocal microscopy. Gastric mucosa of anesthetized rats was superfused with Krebs' buffer containing 0.01 mM NERF (pH-sensitive dye) and 0.5 mM Lucifer Yellow (pH-insensitive reference dye), and excited by a 488 nm laser. (A) Confocal reflectance image of the gastric surface epithelium, with openings of single gastric pits (p) and single surface cells detected. (B) Confocal reflectance image perpendicular (in the XZ plane) to image (A), demonstrating the adherent mucus gel layer (g), apical surface (s) of the epithelial cells, the mucosa (M), and the gastric lumen (L). (C) XZ plane emission ratio image of Lucifer Yellow/Cl NERF (650 nm/575 nm) with pH 3 superfusion. The juxtamucosal mucus gel is alkaline. (D) The same preparation, superfused with pH 5 solution now shows an acidic juxtamucosal pH.

One of the leading assumptions about the role of gastric mucus is that proton flux across it is asymmetric, allowing the unimpeded outward flow of secreted acid, but impeding inward-diffusing luminal protons. Data supporting the presence of viscous fingers has already been presented, which is one means by which asymmetric transport could be achieved. Other suggested mechanisms include transmucous Na⁺/H⁺ exchange, in which an opposing flow of sodium ions facilitate acid secretion, and recently, proton trapping by secreted mucus particles, with acid liberated by pepsinogen. All of these theories, however, remain to be tested in vivo.

One of the most striking findings, however, is that asymmetric transport mechanisms need not be hypothesized. The inward proton flux (back-diffusion) in the rat when luminal pH = 1, as discussed above, is 0.008 µmol/cm²/sec. The outward flux of protons during maximal acid secretion is also 0.008 µmol/cm²/sec in the rat. Similar measurements are also present in dogs. Thus, unidirectional proton fluxes across gastric mucus are equal, suggesting that secreted acid is able to freely diffuse across the mucus layer without the necessity for hypothesizing specialized, asymmetric transport mechanisms. Our in vivo confocal data presented above is consistent with simple diffusion of secreted acid across the mucus, since, when superfusion is stopped, a continuous 'acid front' rapidly extends across the mucus layer, with no evidence, in hundreds of observations, of any inhomogeneity.
Fig. 2 Evolution of concepts regarding the proton barrier function of gastric mucus. (A) Prior to the 1990s, asymmetric transport of protons was assumed, with secreted acid freely traversing the layer by an unknown mechanism, whereas diffusion of back-diffusing acid was retarded by mucus to some extent. (B) After 1990, the concept of viscous fingering of secreted acid was supported by newly published data. A transgel pH gradient was also firmly supported by the available data. (C) With the advent of direct, non-invasive in vivo measurement of mucus pH, the most plausible explanation of the available data is that secreted acid freely crosses the mucus gel by simple diffusion. The modest retardation of proton diffusion through mucus damps rapid changes of luminal pH at the epithelial surface in order to enable the timely activation of intrinsic epithelial homeostatic mechanisms. Juxtamucosal pH is determined by luminal pH such that bicarbonate secretion predominates with pH 3 luminal contents, whereas acidic secretion predominates when luminal pH is raised to 5.

Conclusions

Gastric mucus presents only a modest permeability barrier to acid diffusion. This characteristic enables secreted acid to freely traverse the mucus layer without specialized mechanisms such as tunnels or viscous fingers. Conversely, although mucus is not rate-limiting for proton back-diffusion, it appears to play an important role in damping rapid changes of luminal pH, providing time for activation of cellular homeostatic mechanisms. Recent non-invasive measurements of mucus pH in vivo strongly suggest that mucus pH is set simply by the pH of the fluid entering the layer. Figure 2 summarizes the evolution of the understanding of the barrier function of gastric mucus.

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References


