Regulation of Intracellular pH: Role in Gastric Mucosal Defence

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Abstract. The gastric mucosa is constantly exposed to conditions that would normally be damaging to living cells. A complex defensive system has evolved that involves multiple mechanisms arranged in a laminar fashion, that as a whole constitute the gastric mucosal barrier to acid. As antisecretory therapy becomes perfected, more attention has been focused on these defensive components of the gastric mucosal barrier in disease. Recently, our laboratory has developed a means of measuring intracellular pH (pHi), mucosal blood flow, acid secretion, surface cell acidification rate, and acid secretion simultaneously in vivo. This system has enabled our laboratory to explore how the different components of the gastric mucosal barrier interact so as to protect the pHi of the surface cells under a variety of conditions. Analysis of these studies has revealed a significant inverse correlation between the initial fall in pHi of surface cells during luminal acid exposure and the thickness of the mucus gel, suggestive of a role of adherent gastric mucus in retarding the permeation of luminal protons into the epithelial cells. Another correlation has been between recovery of pHi and the presence of a hyperemic response to luminal acid, which suggests that the hyperemic response is an important defense mechanism in the intact mucosa. Our data is consistent with the hypothesis that gastric mucosal defense mechanisms, like gastric acid secretion, are dynamically regulated according to need. Disturbance of the regulation of these mechanisms, for example by cirrhosis, might be one of the major factors underlying clinical ulcer disease. (Keio J Med 45 (3): 155-160, September 1996)

Key words: stomach, intracellular pH, epithelial cells, mucosal blood flow, mucosal defense mechanisms

Introduction

One of the longest studied mysteries of physiology concerns the question of how the gastric mucosa remains intact and undamaged despite being regularly exposed to amounts of hydrochloric acid which are highly toxic to living cells. Investigations of this question have revealed that a range of defensive mechanisms are present which help mucosal cells to resist acid. A breakdown of these mechanisms would result in an irrevocable fall in surface cell intracellular pH (pHi), cell death, and resultant mucosal injury. It is thus logical that, in the absence of frank injury, the central focus of mucosal defense mechanisms is the maintenance of epithelial cell pHi, which must be preserved despite the enormous H⁺ gradient between blood and lumen. Surface cells can maintain pHi in the presence of luminal acid by slowing proton entry, which is accomplished by enhancing pre-epithelial factors (mucus gel thickness and bicarbonate secretion), epithelial factors (proton and bicarbonate exchangers, intercellular tight junctions) or post-epithelial factors (blood flow, vascular bicarbonate delivery, parietal [alkaline tide] bicarbonate production during acid secretion). It is likely that all of these factors constantly interact in an integrated, coherent fashion in the normal mucosa so as to preserve surface cell pHₚ.

In this review, we will discuss the measurement of pHᵢ of gastric epithelial cells as it relates to protection against acid-related injury. We will describe an experimental system that has been developed in our laboratory as a means of evaluating, and of studying the interactions amongst, gastric defense mechanisms. An example of disordered integration of defense mechanisms, cirrhotic gastropathy, will then be described.
Mechanisms of pH Regulation in Gastric Epithelial Cells

The concept of acid back-diffusion postulates that luminal acid diffuses through the mucus overlying the gastric epithelium, into the interstitial spaces between cells, and eventually into the epithelial and submucosal cells. By this mechanism, irreversible acidification of the epithelial cells is the first indication of injury to the gastric epithelium, which can then manifest as injury or ulcer. The study of gastric mucosal defense mechanisms has thus either been accomplished by measuring the ability of the mucosa to resist injury, which is complicated by the necessity of using a second agent or perturbation to induce injury, and the inability to control aggressive factors (acid and pepsin). Alternatively, measurement of “barrier function”, namely the ability of the mucosa to resist acidification, is an attractive means of assessing mucosal resistance to acid without the above-noted drawbacks of injury studies. Measurement of intramural pH, subepithelial pH, acid back-diffusion, transmucosal potential difference and pH, in vitro and in vivo have all been used to study barrier function. The following summarizes some of the current thinking to date (for review, see refs 1 and 2).

Intracellular pH regulation in gastric surface cells

The gastric surface epithelial cell can be described in terms of the paradigm of bicarbonate-secreting cells that are found in the stomach, small intestine, colon, liver, and pancreas.1 These cells secrete vascularly-derived bicarbonate (which in turn arises from systemic metabolism and from parietal cell acid secretion) by an asymmetric mechanism involving uptake at the basolateral membrane by a sodium-bicarbonate cotransporter and exit across the apical membrane via a chloride-bicarbonate exchanger. Sodium-proton exchange is additionally present on the basolateral, but not apical membrane. Arranged in this fashion, these transporters subserve three important cellular functions: 1) disposal of exogenous protons diffusing into the cells from the interstitial space; 2) excreting excess acid produced as a result of cellular metabolism; and 3) causing the transcellular movement of bicarbonate.3,4 A proton-impermeable apical membrane and an absence of apical-membrane transport proteins with an affinity for protons protects the cell from acidification due to luminal protons.

The physiological consequences of this arrangement of transporters are twofold. The first is that acidification of surface cells is much easier to achieve from the basolateral than from the apical pole of the cell.5 This asymmetry probably results from the increased permeability and surface area of the basolateral membranes to protons.6,7 This enhanced ability to acidify the cells from their basolateral pole has suggested the scheme depicted in Fig 1, wherein luminal protons diffuse through the mucus gel, intercellular tight junctions, and into the surface cells via the basolateral membranes. The second consequence of the arrangement of transporters is one with potential clinical ramifications. Numerous studies have shown that maintenance of pH, bicarbonate secretion in gastric epithelial cells requires exposure to basolateral bicarbonate. This effect has been demonstrated in in vitro studies, in which serosal bicarbonate, but not other buffers, enabled gastric epithelial cells to maintain pH, when exposed to exogenous acid.4,5,8 The same protective effect of vascular bicarbonate has also been demonstrated in ex vivo pouch preparations, where intravenous bicarbonate infusion was correlated with protection from mucosal injury due to hemorrhagic shock and bile acid.9,10 In the absence of exogenous bicarbonate, maintenance of systemic pH by hyperventilation failed to protect against injury. In a dog model of stress gastritis, correction of systemic acidosis was considered more important in the prevention of gastric lesions than was the maintenance of gastric mucosal blood flow.11 These
experiments not only support the role of systemic bicarbonate in the protection of the gastric mucosa, but also are consistent with the strong linkage between mucosal protection and preservation of epithelial cell pH during acid challenge.

**Measurement of pH, in vivo**

Measurement of pH, in vitro has been accomplished with the use of fluorescent, trapped intracellular dyes, that work by a pH-dependent shift in the excitation or emission spectra. The same principles apply to in vivo measurement of pH, which is accomplished by coupling in vitro techniques to the in vivo pH measurements. Kaneko and co-workers reported that they could measure pH of rat gastric surface cells with the fluorescent dye 5,6 carboxyfluorescein diacetate by superfusing the everted gastric mucosa with dye-containing buffer.12 In this manner, the effects of luminal acid and other factors could be assessed in vivo. An additional benefit of this system was that the thickness of the overlying gastric mucus gel could be simply measured by alternately focusing on the surface of the adherent mucus, as delineated by carbon particles, and on the fluorescent surface cells. Mucosal blood flow and acid output were also measured in this system, enabling the investigators to assess the function of the pre-epithelial, epithelial, and post-epithelial components of the gastric mucosal barrier in vivo (Fig 2). The control of luminal pH in this system, regardless of the amount of acid secretion enables study of gastroprotective mechanisms in which aggressive factors are controlled.13,14 Further advantages of the system are that the ability to obtain a measure of gel proton permeability, and to observe the interaction of cell acidification with gastric mucosal blood flow.

**Enhancement of Gastric Defence Mechanisms by Secretagogues**

Since acid is accepted to be the primary agent of gastric injury, it is logical to postulate that gastric defenses are enhanced during stimulation of luminal acid so as to balance defensive capabilities with aggressive factors. Silen's group demonstrated that histamine stimulation of acid secretion by frogs and rabbits increased pH of gastric mucosal cells, increased intramural pH, and decreased transmural potential difference (a measure of mucosal integrity), suggesting that epithelial defense mechanisms were enhanced by stimulation of acid secretion.16-18 More recent experiments have shown that frog oxynticopeptic and parietal cells are much more resistant to acidification from luminal acid when they are stimulated to secrete.4,19 Recently, Taché and colleagues have shown that the centrally administered thyrotropin-releasing hormone (TRH) analog, RX 77368, a vagus nerve-dependent acid secretagogue, is capable of protecting the gastric mucosa from damage due to 60% EtOH.20-22 Further studies with centrally administered RX 77368 have suggested that gastroprotection is mediated by prostaglandins20-23 and is associated with increased gastric mucosal blood flow.24,25 Gastrin and histamine have been shown to be gastroprotective against experimental gastric injury.26-31

The mechanisms of secretagogue-associated gastroprotection have been further explored with in vivo microscopy. The synthetic gastrin analog pentagastrin as well as vagal stimulation accomplished with central TRH administration increased mucus gel thickness, and unexpected effect of acid stimulation, suggesting up-regulation of pre-epithelial factors during acid secretion. Both secretagogues markedly enhanced pH homeostasis during acid superfusion, manifest as a significantly lower initial acidification rate and as a recovery of pH towards baseline, and were associated with an acid-induced sharp increase in mucosal blood flow.13 The gastroprotective mechanisms of the secretagogues differed, in that gastroprotection associated with vagal (TRH) stimulation was diminished by prostaglandin synthase inhibition with indomethacin, whereas the effects of pentagastrin were not.32 The studies also demonstrated that most of the gastroprotective effects of pentagastrin occur through the release of histamine.13 These studies show that secretagogues improved pH homeostasis during luminal acid challenge due to the enhancements of pre-epithelial, epithelial and post-epithelial defense mechanisms, and that prostaglandins and histamine play a significant role in this pH improvement. Gastric defense mechanisms are thus dynamically regulated according to the acid-secretory state of the gastric mucosa, balancing aggressive and defensive factors.
Interaction between Blood Flow, pH, and Mucus Gel Thickness

In the past few years, the ability to simultaneously measure components of the pre-epithelial, epithelial, and post-epithelial layers of the gastric mucosal barrier in vivo has yielded information on how these components interact in vivo in response to pharmacologic and physiologic manipulations. Two intriguing findings concern the interaction of blood flow and pH, and the relation between the initial acidification rate and mucus gel thickness. The ability to correlate these measurements has indicated that there is an unexpectedly complex interaction between components of the gastric mucosal barrier.

Blood flow and pH

Nishizaki and co-workers recently discovered that blood flow and pH were temporally related in that a fall in pH during luminal acidification triggered a hyperemic response to acid, which in turn was associated with an enhanced recovery in pH. This phenomenon was observed after treatment with luminally-applied isoproterenol, during pentagastrin infusions, and after treatment with a topical prostaglandin analog. The recovery of pH with the hyperemic response suggested that this may be one of the means by which enhanced gastric mucosal blood flow prevents gastric injury, and why prevention of the hyperemic response by denervation or other means enhances the susceptibility of the mucosa to injury (Fig 3).

Mucus gel thickness, initial acidification rate, and the role of mucus

Several studies conducted in vitro have shown that gastric mucus can slow proton diffusion and can enable the formation of a pH gradient across the mucobicarbonate layer. The role of mucus in the protection of the gastric mucus, however, has been controversial. Slowing of proton diffusion by mucus does not affect, for example, the amount of protons entering the epithelium over time if luminal proton concentration is held constant. Several recent studies that have been designed to address this issue have yielded data consistent with a protective role of mucus. For instance, simultaneous measurements of intracellular pH and the thickness of the mucus gel overlying gastric surface cells in vivo indicated that surface cell acidification rates and mucus gel thickness were inversely related. In other words, the thicker the mucus gel, the slower the acidification of the surface cells. Such a relationship is depicted in Fig 4. This relationship is consistent with the hypothesis that proton permeation is slowed by gastric mucus. Other studies indicated that a physico-chemical change of the mucus barrier that slowed proton permeation, produced in the absence of measurable changes in mucosal blood flow, acid secretion, or gel thickness, was associated with protection against injury due to nonsteroidal anti-inflammatory drugs (NSAIDs). These findings, coupled with the thicken-

![Fig 3](image-url)
Fig 4 Relationship between pH and mucus gel thickness. This figure depicts the results of 29 separate experiments in which rats were treated with substances which either increased, decreased or did not affect mucus gel thickness. Note that the two variables are inversely related, suggesting that the gastric mucus impedes proton permeation in vivo. Adapted from Engel E et al.15

Gastric Defense Mechanisms in Cirrhosis

Portal hypertensive gastropathy is a complication of cirrhosis with portal hypertension. The disease is associated with a spectrum of gastric lesions, ranging from diffuse erythema, through a reticulated “snake skin” pattern and “cherry-red” spots, to frank hemorrhage.40,41 Portal hypertensive gastropathy is the source of hemorrhage in most patients with cirrhosis of the liver with non-variceal upper gastrointestinal bleeding, and commonly complicates sclerotherapy of esophageal varices.42 The pathogenesis of portal hypertensive gastropathy remains unclear, although increased gastric mucosal blood flow with microvascular congestion and resultant dilation of microvessels and development of vascular ectasias have been implicated.40,41 There is little evidence to suggest that aggressive factors such as acid secretion are involved, focusing attention on gastric defensive factors such as the pre-epithelial mucus-bicarbonate layer.43,44

There are very few studies that have examined the effect of experimental cirrhosis on pH regulation in gastric surface cells. Nishizaki and co-workers demonstrated that gastric defenses were impaired in carbon tetrachloride (CCl4) induced cirrhosis of the liver, as manifest by spontaneous gastric mucosal lesions, impaired gastric surface cell pH homeostasis, a thin mucus gel layer, and decreased gastric mucosal blood flow.45 Cirrhosis also impaired the pentagastrin-associated hyperemic response to acid, suggesting damage to the neural mechanisms associated with this response. Cirrhosis thus affects the pre-epithelial and post-epithelial components of the mucosal barrier, impairing the ability of the surface cells to maintain pH during acid exposure. This impairment of pH homeostasis was associated with an enhanced susceptibility to mucosal injury.

Summary and Future Directions

We have shown that the measurement of pH during luminal acidification is a sensitive early measure of the state of gastric mucosal defense mechanisms. This technique, combined with measurement of mucus gel thickness, acid secretion, and mucosal blood flow, has enabled us to understand how gastric defensive mechanisms integrate into a coherent, laminar defensive system, that varies according to the presence of luminal acid and the acid-secretory state of the mucosa. Interference with one or more of these mechanisms is associated with pathologic conditions such as cirrhotic gastropathy. On the other hand, pharmacological enhancement of one or more defensive factors can increase the resistance of the gastric mucosa to acid-induced injury.

Recent data has suggested that the two most common entities associated with peptic ulcers, namely H. pylori and NSAIDs produce ulcers by impairing defense mechanisms rather than by enhancing aggressive factors.46 Further study of gastric defensive mechanisms should be fruitful for those devising new treatments for these vexing and common problems.

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References


32. Tanaka S, Kaunitz JD: Indomethacin does not alter the effect of pentagastrin on rat gastric defense mechanisms. Peptides 1995; 17: 155–159


