YALE JOURNAL OF BIOLOGY AND MEDICINE 72 (1999), pp. 125-131.

Acid and the Esophagus

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INTRODUCTION

Most of what we know about gastroesophageal reflux disease (GERD)[b] suggests that it is caused by disordered neuromuscular function in the esophageal body and in the lower esophageal sphincter (LES) separating it from the stomach. This implies that keeping gastric contents out of the esophagus in patients with reflux should restore normality and cure GERD. However, for most of this century, successful therapy of GERD has usually been based on reducing acidity of the gastric (and hence esophageal) contents and ignoring neuromuscular dysfunction.

Much effort has been expended on trying to distinguish between direct injurious effects of acid on the esophageal mucosa and indirect effects due the effects of acid on some other component of the refluxate, rendering the latter more or less injurious under conditions of varying acidity. Despite the considerable success of potent proton pump inhibitors (PPIs) in treating GERD, there is in every treatment trial a group of 5 to 15 percent of those admitted who continue to have heartburn despite virtually complete suppression of gastric acid secretion. This article will review what we know about refluxate-medicated injury to the mucosa over the pH range 1.0 to 8.5.

EXPERIMENTAL STUDIES

Taking off from the work of Wangensteen in the 1950s [1-3], Redo and colleagues performed the seminal studies of the modern era in 1959 [4]. In isolated esophageal segments in dogs, they showed that perfusing with strongly acidic gastric juice (pH 2.10) led to ulceration in 88 percent of animals, but when pH was between 2.10 and 3.0, injury was reduced; at pH > 3.0 there was little or no injury. When peptic activity of the juice was reduced or abolished by 0.1 N. NaOH and reacidification to pH 2.1, or by boiling prior to use, there was no injury. Similarly, perfusion with dilute hydrochloric acid alone (pH $\geq$ 2.0) or acid plus 0.1 percent pepsin caused no injury.

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[b] *Abbreviations:* GERD, gastroesophageal reflux disease; PPI, proton pump inhibitor; DGER; duodeno-gastro-esophageal reflux; EM, electron microscopy; PD, potential difference; RE, reflux esophagitis.

However, when pepsin concentration in the same perfusate was raised to 2 percent, severe ulceration developed in over 80 percent of animals and esophageal perforation in 15 percent. All regions of the esophagus were similarly sensitive to injury.

Paradoxically, when pepsin concentration was raised to 10 percent, injury was much reduced at pH of less than 2.0, and absent at higher pH. Perfusing these segments with pure human bile, human pancreatic juice or mixtures of these together or of bile plus gastric juice produced little or no injury. From these studies pepsin seemed to be the major agent of injury, but the injury appeared to depend both on pH and pepsin concentration. Furthermore, dialysis of gastric juice prior to perfusion studies greatly increased its capacity to cause injury, suggesting that dialysis had removed either a pepsin-inhibitor or some endogenous protective substance(s) present in the juice, e.g. prostaglandins or phospholipids. Similarly, addition of bile to gastric juice reduced injury. These observations still remain unexplained.

This work was confirmed and extended by Goldberg and Dodds [5], using porcine pepsin in the cat esophagus perfused for 1 hour. At pH 1 and pH 1.3, acid alone caused esophagitis. At pH above 1.3, acid alone caused no injury, but addition of pepsin caused immediate injury maximum at the pH optimum of the enzyme (1.3 to 1.6). When the pepsin inhibitor Depepsin was added to the perfusate, no injury occurred. Since esophageal pH is usually above 2.0 in GERD patients, these studies supported the notion that acid acted by activating pepsinogen to pepsin, leading to proteolytic degradation of the mucosal surface. This, however, does not exclude the possibility that, in the mucosa thus damaged, back diffusion of acid exacerbated the injury; at high acid concentrations, pepsin was not essential to producing injury.

Over the next 15 years, initial optimism about the effectiveness of cimetidine in GERD gradually gave way to the realization that in many cases even quite high doses of the drug produced little or only transient symptom relief. Furthermore, over the previous 25 years there had been a growing realization that heartburn and GERD occasionally occurred in the virtual absence of gastric acid, as in patients with pernicious anemia or with achlorhydria following gastric surgical procedures.

This prompted a resurgence of interest in the contributions of bile salts and alkaline reflux to esophagitis by workers at Walter Reed Army Hospital [6-8]. Studies by Harmon et al. [6] clarified some important questions about bile salts. In the perfused rabbit esophagus (without pepsin) they showed that at pH 2.0 conjugated bile acids (salts) disrupted the mucosal barrier while unconjugated bile acids did not. However, at pH 7.0, unconjugated bile acids also disrupted the barrier. They established that when the perfusate pH was less than the pKa of the bile acid it precipitated and caused no damage; however, when pKa was less than the pH of perfusate, the bile acid solubilized and caused injury. The studies revealed that the mucosa was very sensitive to bile acids in solution (as low as 3 mM disrupted the barrier) and showed no ability to recover from such injury [6].

Next, using a continuously perfused rabbit esophagus, they studied the potentially injurious effects of trypsin, taurodeoxycholate and pepsin at pH 7.5 while measuring both macroscopic mucosal injury and esophageal mucosal barrier function [7]. The latter was assessed by measuring net fluxes of hydrogen ion, potassium, glucose, water and hemoglobin into/out of the perfused lumen. As judged by pathological injury, trypsin caused the most severe mucosal damage and bleeding (at close to its pH optimum, 8.1) but caused only minimal alteration in barrier

function; pepsin or test solution caused no injury at this pH.

In contrast, taurodeoxycholate, which caused little apparent mucosal injury, caused profound disruption of barrier function in the apparently uninjured mucosa, with marked leakage of hydrogen ions out of (and potassium into) the lumen. Because the normal pH in the esophageal lumen is 5 to 6, and because reflux of acid or alkali is minimal and rapidly cleared under normal conditions, no injury occurs with transient reflux in normal subjects. However, reflux of acid-plus-activated-pepsin or alkali-plus-activated-trypsin (potentially derived from pancreatic juice) can cause considerable injury if not rapidly cleared. While the injury caused by pepsin occurs only in the presence of acid, that of trypsin can occur in its absence and causes no leakage of hydrogen ions into the mucosa. The studies also revealed that considerable damage to the mucosa — as judged by grossly impaired barrier function — could occur in the absence of macroscopic injury or changes revealed by light microscopy.

In subsequent studies [8], the same group showed that the addition of taurodeoxycholate increased the injury caused by trypsin at pH 7.5 in a dose-dependent manner, while its addition to a solution of pepsin at pH 2.0, dose-dependently reduced both the gross and microscopic injury caused by acid-pepsin alone and also reduced barrier dysfunction. Lysolecithin can be generated *in vivo* from the action of pancreatic phospholipase A on biliary lecithin. Perfusion of the esophagus with lysolecithin also damaged the esophageal mucosa in the presence but not in the absence of acid [9], the damage increasing with acid concentration. From all these observations, it appeared that agents such as pepsin, lysolecithin and conjugated bile salts in solution, caused esophageal mucosal damage in the presence of acid, partly by increasing back-diffusion of hydrogen ions into the mucosa and partly by direct damage to cells, but that not all such damage was apparent to the naked eye. However, the effects of bile salts at acid pH seemed to vary with their pK values, with whether they were conjugated or unconjugated, and in the presence or absence of pepsin.

On the other hand, the esophagitis caused by alkaline reflux was trypsin-mediated and exacerbated in the presence of bile reflux, particularly when bile acids were unconjugated. Furthermore, surgical diversion of trypsin and duodenal contents away from the esophagus cured the heartburn associated with partial gastrectomy even in the continued presence of pepsin [10]. Despite these animal and human observations, there remained some uncertainty as to the extent to which duodeno-gastro-esophageal reflux (DGER) contributed to acid-pepsin-mediated esophagitis in intact humans. At about the same time, Hirschowitz [11], in studies in 155 patients with esophagitis and 508 appropriate controls, concluded that neither the composition of gastric juice in terms of acid or pepsin, nor the basal or stimulated outputs of these substances, could be correlated with the presence or severity of esophagitis.

In the past five years, a new fiberoptic system (Bilitec 2000, Synectics, Inc. Irving, TX; now Medtronics, Inc.) was developed that measures gastric and esophageal concentrations of bilirubin (and by implication the amount of DGER) spectrophotometrically, independent of pH [12]. Taking total time at pH less than 4.0 as an indicator of acid reflux, and total time with bilirubin absorbance at more than 0.14 as an indicator of DGER, in 30 patients with GERD and in 20 controls, it was found that both parameters increased across a spectrum of increasingly severe GERD. Exposure to both acid and DGER was the most prevalent pattern in 100 percent of patients with complicated and 89

percent of those with uncomplicated Barrett's esophagus; in 79 percent of patients with esophagitis; and in 50 percent of GERD patients without esophagitis. These criteria were not met in controls [13].

In patients there was a strong correlation between percent time at pH < 4.0 and percent time with bilirubin absorbance > 0.14 ($r = 0.73$: $p < .01$). These data indicated that acid (with pepsin) and DGER occur simultaneously in the majority of GERD patients. More recent studies in 32 partial gastrectomy patients [14], using the same methodologies, showed that such patients formed a heterogeneous group: 28 percent had mixed reflux (acid +/DGER+), 50 percent had only DGER (acid-/DGER+), and 22 percent had neither acid nor DGER (acid-/DGER-). Both mixed reflux and DGER groups had GERD symptoms. Patients in the (acid+/DGER+) mixed group had esophagitis, but no (acid-) patient had esophagitis, regardless of the presence or absence of DGER. DGER alone in the absence of acid (acid-/DGER+) caused heartburn but not esophagitis. Although there are undoubtedly some post-gastrectomy patients in whom esophagitis can be attributed to trypsin and bile salts [10], in the majority of those with esophagitis acid is present; DGER did not correlate with mucosal injury [14]. Furthermore, because of effects on the volume/output of gastric secretions, proton pump inhibitor therapy greatly reduces DGER in GERD patients [12].

These data support the clinical practice of first treating post-gastrectomy reflux with a PPI drug, and only if this fails considering detailed investigations of intraesophageal contents and pH, as a prelude to diversionary surgery. "Alkaline reflux" appears to contribute to heartburn but not esophagitis. From the finding of esophagitis only in the acid+/DGR+ group, there appears to be a requirement of acid for most visible mucosal injury. From what we know, the acid would activate pepsin, inactivate trypsin and aid in causing mucosal injury by lysolecithin and conjugated bile salts, although the latter may also result in some inactivation of pepsin. Thus, we return to the problem of separating the direct effects of acid from the indirect effects that it mediates through interactions with other substances, such as pepsin, bile salts and lysolecithin. From all of the above considerations, it appears unlikely that the effects of acid are confined solely to the activation of pepsin.

What then do we know of esophageal injury or dysfunction caused by acid alone? Since 1977, Orlando and co-workers have relentlessly pursued this question. Starting with comparative studies in several species, they showed that the transmucosal potential difference (PD) and the structure of the stratified squamous epithelium were very similar in man and rabbit, justifying the choice of the rabbit as a useful animal model for studying human disease [15]. They next showed that continuous perfusion of the esophagus with acid led to a progressive decline in PD and a progression of cellular and epithelial injury, beginning with swelling of the intercellular spaces, followed by cell swelling and rupture, and ultimately epithelial disruption in the area closest to the entry of acid [15]. Early in injury, while water is accumulating between cells on electron microscopy (EM), mucosal electrical resistance falls, but sodium transport is not impaired. Later, with progression of injury, sodium transport is abolished, followed by marked changes on EM but no macroscopic change or transmucosal necrosis. The addition of dilute pepsin to the perfusate accelerates the changes but does not change the type or extent of injury [17]. The injury progressively moves from the stratum corneum, to the stratum spinosum but rarely deeper than this. The pattern of injury, rate of progression and interference with Na^+/K^+ATP-ase

activity and sodium transport are all dependent on acid concentration [18]. At lower concentrations (20 to 40 mm), and early in injury, there is increased diffusion of H^+ from lumen to tissue to blood. Later, and at higher acid concentrations (80 to 120mm), loss of ATP-ase activity results in cell swelling and rupture as in [17].

These observations raised questions as to what structural components of squamous epithelium formed the barrier responsible for resistance to the diffusion of acid into tissues. In extensive microscopic studies employing both EM and histochemistry [19], the group showed that acid caused disruption neither of the "tight junctions" or desmosomes, nor of intercellular lamellar lipid bodies, but instead seemed to cause destruction or solubilization of the dense glycoconjugate material secreted by cells into the intercellular spaces. Later, endoscopic biopsies of the human esophagus, from controls and from patients with heartburn, were examined by transmission EM. They showed that dilated intercellular spaces larger than 2.4 μm were present in 73 percent of heartburn patients and in no controls [20]. This finding did not differ in patients with or without visible or histologic erosive esophagitis.

In their most recent studies [21], the development of symptoms during perfusion of the esophagus with 0.1 N. HCL was correlated with measurements of PD (a measure of barrier function, previously correlated with changes in intercellular spaces) in four groups of humans: healthy controls; those with endoscopy-negative GERD, before and after omeprazole therapy; patients with reflux esophagitis (RE); and GERD patients in remission post-fundoplication. The findings were that all patients with esophagitis developed symptoms during acid perfusion, as did 60 percent of those with endoscopy-negative GERD before therapy, reduced to and 30 percent during therapy. Two of 10 surgically-treated patients developed mild symptoms, but none of the controls did. Although baseline PD measurements differed in the four groups (lowest in controls, -12mv; highest in RE, -17mv), PD rose similarly during acid infusion in all four groups to an extent comparable to the "early or mild barrier injury" changes observed in animal studies [18].

These data suggest that symptoms accompany mild barrier injury during acid infusion only in subjects with some pre-existing tissue injury, as revealed by EM or baseline PD measurements, and that such changes are more sensitive and specific for acid injury than are endoscopic findings on naked eye examination or "on conventional microscopic examination of mucosal biopsies." They also raise the possibility that trans-esophageal measurements of PD, combined with the PD response to acid infusion, might be sensitive indicators of subclinical esophageal injury occurring within the range of "normal" reflux as defined by 24-hour pH-metry.

Subclinical injury brings us to the final issues with regard to acid and the esophagus, namely afferent sensitivity, pain threshold and patient responsiveness. In studies of non-referred populations of heartburn sufferers, esophageal symptom scores correlate well with percent time at $pH < 4.0$ for the total and upright, but not the supine, time intervals [20]. These 24-hr pH results were not predictive of the response to minimum doses of H_2-antagonists [22]. Studies by Trimble et al. [23] showed that esophageal sensory responses to balloon distension were significantly increased in those with symptomatic but not excessive (by 24-hr pH) gastroesophageal reflux, compared to those with obvious excessive reflux or to asymptomatic healthy controls. This indicated that some heartburn patients without esophagitis had a low threshold for painful sensation. In contrast, responses to

balloon distension were reduced and sensory thresholds increased significantly in esophagitis patients with Barrett's Esophagus compared to the same controls [23). Interestingly, although the stimulus was mechanical and not chemical, 55 percent of patients in the hypersensitive group described the balloon-evoked sensation as identical to their GERD presenting symptoms.

In more recent studies in normal volunteers, the pain threshold for responses to electrical stimulation in the mid-esophagus was lowered by distal infusion of acid. Since acid did not enter the segment under study, sensitization and hyperalgesia must have occurred centrally [24]. However, work from other centers suggests that the afferent sensory thresholds vary with the type of stimulus supplied, the type of sensation evoked and the presence or absence of pre-existing disease or injury in the subject [25]. This work is in its early stages but is very important. Results will hopefully be correlated with changes in PD and with EM appearances of the tissue.

CONCLUSIONS

There is increasing evidence that the effects of acid in the esophagus can be grouped as direct and indirect. The presence of esophagitis as recognized endoscopically or by conventional histology seems to reflect the presence of more advanced GERD, contributed to by indirect effects of acid on the damage mediated by pepsin, conjugated bile salts, lysolecithin and perhaps other substances. These gross changes may be superimposed on subclinical, but functionally significant, direct injury by acid to the mucosa. There is abundant evidence based on studies of PD, ion fluxes, H^+ and Na^+ transport, Na^+/K^+ ATP-ase activity, electron microscopic and histochemical studies that acid alone causes significant but often clinically occult esophageal mucosal injury. This last may contribute to heartburn in patients in whom 24-hour pH studies fail to show results that meet conventional criteria for reflux. Whether such subclinical injury also contributes to disordered sensation, altered pain thresholds, hyperalgesia and dysmotility remains to be seen.

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