Dilated Intercellular Spaces as a Marker of GERD

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Gastroesophageal reflux disease (GERD) is typically heralded by the substernal burning pain of heartburn. On endoscopic examination, about one third of GERD subjects with heartburn have erosive disease, and the remainder have nonerosive reflux disease (NERD). Unlike patients with erosive disease, those with NERD (~ 50%) often do not respond to therapy with proton pump inhibitors (PPIs), raising the question of whether they have NERD and, if they do, whether the cause of their symptoms is similar to those who respond to PPIs. Recently, biopsies established that subjects with heartburn and PPI-responsive NERD, like those with erosive esophagitis, have lesions within the esophageal epithelium known as dilated intercellular space (DIS). In this article, we discuss the physicochemical basis for DIS in acid-injured esophageal epithelium and its significance in GERD. Although DIS is not pathognomic of GERD, it is a marker of a break in the epithelial (junctional) barrier reflecting an increase in paracellular permeability.

Introduction

Gastroesophageal reflux, the orad movement of gastric contents into the esophagus, is a normal phenomenon that occurs multiple times daily. Gastroesophageal reflux disease (GERD) occurs when this process becomes pathologic, leading to tissue injury in the esophagus, oropharynx, larynx, or respiratory tract. The difference between those with normal reflux and those with reflux disease is the failure of the esophageal defense system, which is composed of three different mechanisms: limiting the frequency of reflux, limiting the duration of contact between the epithelium and acid, and the ability of the tissue to directly resist injury by acid [1]. The mechanisms that limit acid contact time with epithelium include the antireflux barriers (ie, lower esophageal sphincter and diaphragm), and the luminal clearance mechanisms (ie, gravity, peristalsis, and the buffered secretions of the salivary and esophageal glands). By decreasing the contact time of acid with the epithelium, these mechanisms give tissue the time to circumvent injury to the esophagus and to the extraesophageal organs. At the tissue level, the mechanisms that protect against injury by acid are referred to as "tissue resistance" and are designed specifically to protect the esophagus during acid contact with the epithelium. Tissue resistance is composed of a mucus- and bicarbonate-containing unstirred water layer, the apical membrane of the esophageal squamous cells, the intercellular junctional complexes (IJCs), intra- and intercellular buffering with bicarbonate, and H^+ ion transporters located on the basolateral membrane [2]. An intact tissue defense system can resist the effects of acid contact for at least 30 minutes. This is evident clinically because the Bernstein (esophageal acid perfusion) test in healthy subjects does not result in symptoms despite continuously bathing the esophagus in 100-mM HCl for up to 30 minutes [3]. However, when one or more of these defensive mechanisms fail (ie, antireflux barrier, luminal clearance, or tissue resistance), then acid injury can occur, leading to signs and symptoms of GERD.

Lesions Accompanying Heartburn

Damage to the esophagus is the most common manifestation of GERD and is often heralded by the presence of a substernal burning pain known as heartburn. Endoscopically, 60% to 70% of those with heartburn have an esophagus that appears grossly normal (nonerosive reflux disease [NERD]), and the other 30% to 40% have clear evidence of tissue damage with erosions (erosive esophagitis). Both erosive esophagitis and NERD symptomatically respond to acid suppression with proton pump inhibitors (PPIs), although in patients with NERD a response is less likely, with only 50% obtaining symptom relief compared with 60% to 70% in erosive esophagitis [4]. This difference in response to acid suppression has led to an intense debate regarding symptom etiology in NERD: does the heartburn in subjects with NERD arise from a different mechanism (eg, visceral hypersensitivity or sustained

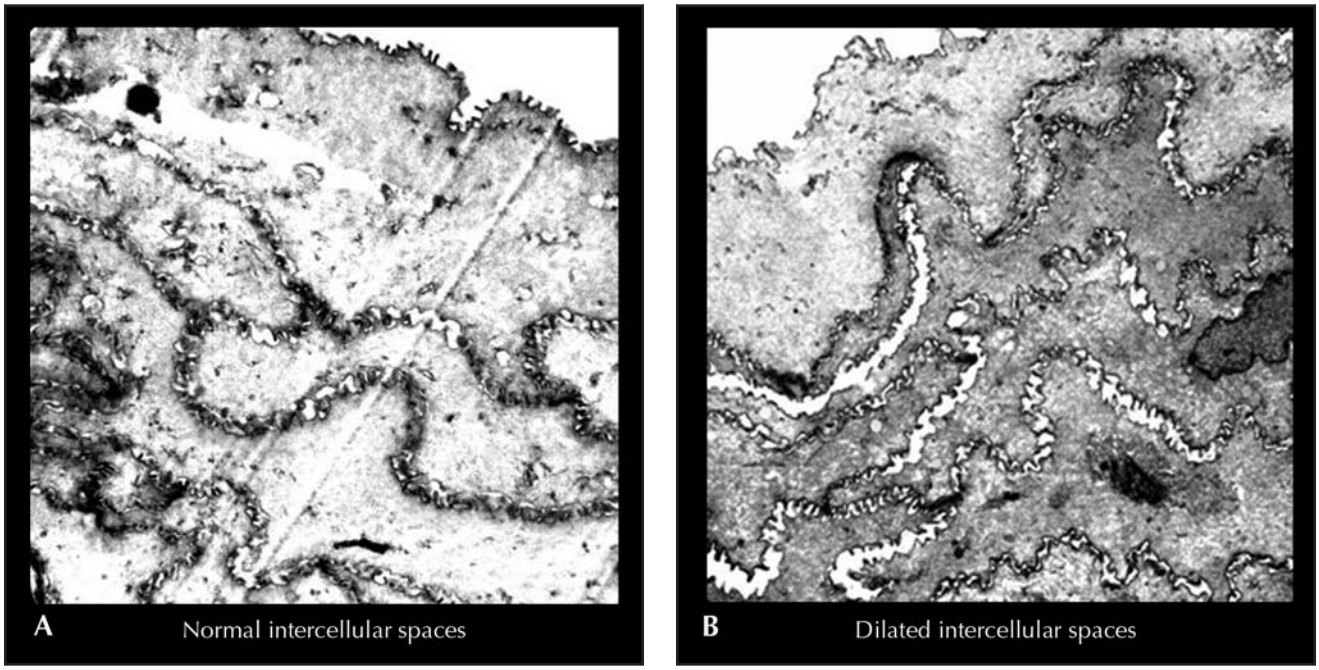

Figure 1. Esophageal biopsies illustrate the appearance of a normal intercellular space diameter in (**A**) healthy epithelium (mean ~ 0.33 μm) and (**B**) epithelium showing dilated intercellular spaces in a subject with nonerosive reflux disease (mean ~ 0.92 μm). (Original magnification × 3000).

esophageal contractions) or do the symptoms arise from a nonreflux mechanism (eg, functional heartburn) [5,6]? Nonetheless, over the past 10 years, the mechanism of the symptom of heartburn in those with PPI-responsive NERD has become clear. On esophageal biopsy, these patients have been found to have a lesion known as dilated intercellular space (DIS) within the esophageal epithelium (Fig. 1) [7]. Thus, if the intercellular space diameter had a value greater than 2.4 μm, there was 100% specificity and a 73% sensitivity as a discriminator between those with NERD and healthy subjects; in the same groups, the mean value (± SE) for subjects with NERD was 1.00 ± 0.15 μm and for healthy subjects was 0.46 ± 0.06 μm ($P < 0.057$). DISs were identified in hematoxylin and eosin–stained esophageal biopsies viewed by light microscopy [8]; however, they are most reliably demonstrated in esophageal biopsies that have been fixed and viewed by transmission electron microscopy (TEM) [7].

Method of Measuring DIS

No standard method exists for determining a mean intercellular space diameter; however, a commonly employed method is examining and photographing the specimen using TEM. Intercellular space diameter is then assessed in the most luminal 10-cell layers, which are recorded from 10 photomicrographs of each specimen. On each photomicrograph, typically magnified 3000 times, the diameter of the intercellular space for each cell layer is obtained in microns (μm) by measuring the length of a line drawn perpendicularly to the neighboring membranes. This yields 10 diameters for each of the 10 photomicrographs, or 100 diameters for each specimen. The 100 values are averaged to provide a mean diameter of the intercellular space for the specimen.

In these specimens, DIS is most prominent in the lower cell layers (ie, the lower stratum spinosum and stratum germinativum [basalis]), although it is also present to a lesser extent within the upper layers of stratum spinosum and stratum corneum. The reason for this difference has not been subjected to rigorous study, but is likely because the squamous cells closer to the surface are more rigid and have less pliable lateral cell membranes. This is exemplified during cell shedding from the epithelial surface (eg, when performing cell separation for culture) because they retain their shape. This increased rigidity limits expansion of the intercellular space by fluid, thus decreasing the capacity of the upper cell layers to separate when compared with the lower, younger, and more pliable cell layers. Another notable morphologic feature in specimens containing DIS is that the intercellular spaces have a scalloped appearance, in which dilated areas appear to be partially compartmentalized because of areas where the lateral membranes remain in close apposition. On closer inspection by TEM, it is evident that these areas of close apposition are maintained by intercellular desmosomal connections.

Physicochemical Basis of DIS

As noted previously, DIS refers to the presence of prominent fluid-filled spaces between cells in the esophageal

epithelium (Fig. 1). Normally, the IJC permits cells to adhere more tightly to each other using bridging protein connections mediated by claudins and occludin for the tight junction, e-cadherin for the adherens junction, and desmoglein and desmocollin for the desmosomes [9,10]. This tripartite junctional complex provides at least a two-staged physical barrier to the passage of acid and other noxious agents between cells as one of the tissue's key structural defense mechanisms. Consequently, when one or more of these bridging proteins fail, the membranes separate and a paracellular "leak" occurs, which leads to generation of intercellular space dilatation (see below). Nonetheless, it is important to appreciate that although DIS is present, the actual adhesion of the ICJs as observed on TEM are not abnormal. Indeed, the best method to determine if DIS is associated with a paracellular leak is to mount the tissues in an Ussing chamber. In the chamber, it becomes obvious that the specimens with DIS have a lower electrical resistance than healthy epithelium (resistance is a marker of permeability) and that molecules from one compartment (ie, lumen side) have a much greater ability to diffuse across the tissue and into the other compartment (ie, serosal or blood side) [11]. In the absence of erosions or cell necrosis, an increase in the flux of molecules like mannitol or dextrans of varying size is consistent with the leak being across the paracellular pathway as opposed to the transcellular pathway.

DIS was first identified independently by Pope [12] in 1978 and by Hopwood [13] in 1979; however, the significance of the lesion remained unclear until 1996, when a clinical study quantified intercellular space diameters in esophageal biopsies of patients with NERD and erosive esophagitis and contrasted the findings to those with a healthy esophageal epithelium [7]. This study effectively established that patients with NERD and erosive esophagitis had DIS, and because both clinical entities had similarly sized spaces, it suggested that they shared a common pathogenetic pathway. Thus, the presence of DIS in NERD would suggest that this lesion likely precedes and therefore promotes the development of erosions. Subsequently, several well-done clinical studies have further clarified the role of DIS in GERD by finding that it was present in patients with heartburn irrespective of the level of esophageal acidity measured by pH monitoring [14], that it resolved in tandem with the resolution of heartburn after PPI treatment [15], and that it could be induced by esophageal acid perfusion in healthy human subjects [16]. Taken together, these data firmly establish that DIS is a histopathologic feature of NERD and erosive esophagitis, can be generated by excess acid exposure, and correlates well with the symptom of heartburn [17].

Even before the demonstration of DIS in GERD, additional information was obtained about the nature of DIS from in vivo and in vitro animal studies. For instance, in 1981 acid-perfused rabbit esophagi were shown to develop DIS that was associated with nonerosive changes on biopsy [18,19]. Moreover, the lesion was associated with an increase in paracellular permeability, a key point when considering the mechanism for symptom generation in humans.

Recently, adding another nuance to this lesion, the physicochemical basis for its generation was elucidated using acid-exposed rabbit esophageal epithelium mounted in Ussing chambers [20••]. Specifically, researchers found that first, high concentrations of luminal H^+ ions were needed to break the epithelial barrier, effectively increasing the paracellular permeability and, second, high concentrations of luminal Cl^- ions were needed to diffuse through the leaky junctions into the intercellular space in sufficient quantities to create an osmotic gradient that would then pull water into the area [20••]. The hydrostatic force resulting from excess water moving into the intercellular space accounted for the separation of adjacent cell membranes and the appearance of DIS. These concepts were supported by experiments showing that dilated spaces did not occur in the presence of low H^+ ion concentrations, balanced Cl^- ion concentrations (adding Cl^- ions to the serosal side to prevent diffusion into the intercellular space), or exposure to sulfuric acid in which, despite the increase in paracellular permeability, spaces do not dilate because the larger sulfate ions are unable to penetrate the junction in sufficiently high concentrations to create the osmotic force needed to separate the membranes.

Although the dilated intercellular spaces are a hallmark of a damaged IJC leading to a leaky paracellular pathway, this process is not static in terms of whether acid-induced symptoms or injury develop. As mentioned earlier, the IJC is only one of the multiple components of the tissue defense system. When the IJC fails and DIS occurs, acid enters the intercellular space in larger quantities. Initially, the acid is buffered by intercellular bicarbonate that diffuses from nearby capillaries and/or is derived from bicarbonate generated within the tissue by carbonic anhydrases [2]; however, when the buffering capacity of the space is exceeded, two things happen. First, the acid-sensitive nociceptors present in the intercellular space are stimulated, leading to the symptom of heartburn [21,22]. Second, intercellular acidification exposes the squamous basolateral membrane to acid. This membrane contains an acid-absorbing, Na-independent Cl/HCO_3 exchanger that, when activated by excess intercellular H^+ ions, results in cytosolic acidity [2]. In turn, this initiates a cascade of events leading to loss of cell osmoregulation, cell edema, and ultimately cell death [23,24]. Cell death is counterbalanced by tissue reparative processes, including restitution and replication. When cell death exceeds the tissue's capacity to repair itself, then erosions appear and erosive esophagitis occurs; however, when tissue repair exceeds the rate of cell death, then the injury remains as a form of NERD.

PPI-resistant Symptoms

The described pathway shows how increased acid contact time or highly acidic gastric refluxate can result in DIS. Because acid is the mediator in this process, these individuals would respond well to acid-suppressive therapy with a PPI; however, this does not explain the presence of PPI-resistant NERD. One possibility was suggested by the fact that some patients with NERD appear to respond to weakly acidic refluxate [25]. Moreover, a recent study suggested that DIS can occur through weak acid-mediated mechanisms, leading to injuries that would not necessarily respond to the level of acid suppression achievable by PPIs. One potential non–acid-mediated mechanism for DIS is an increase in the pathogenicity of the refluxate (other than increased acidity), due to conjugated bile salts (others are alcohol concentrations > 10% and hypertonic solutions) [26,27,28•]. Bile salts are normally present at very low concentrations in the stomach; however, in individuals with duodenogastroesophageal reflux, the bile salt concentration can be much higher. Consequently, experiments have shown that bile salts at acidic and weakly acidic pH are capable of producing DIS [28•]. Although this finding suggests another possible pathway by which reflux can cause PPI-resistant NERD, as noted earlier, other reflux-generated or -independent mechanisms may also be at work in select patient populations. For instance, visceral hypersensitivity and sustained esophageal contractions may be elicited by refluxate volume or gas content [5,6].

Yet another potential non–acid-mediated mechanism for DIS is periods of acute physiologic stress [29]. In a rat model of partial-restraint stress, researchers showed that stress alone could produce DIS, whereas stress coupled with acid-pepsin exposure further increased the magnitude of the lesion. However, stress alone may induce reflux and the animals were not acid suppressed, leaving open the possibility that DIS in this model resulted from acid reflux. Because luminal hypertonicity and possibly physiologic stress can also produce DIS, the presence of the lesion is not pathognomic of acid-induced damage to the esophagus. Nonetheless, regardless of how DIS is generated, its presence is evidence of a broken barrier that can promote the free movement of luminal content into the intercellular space. Consequently, luminal content containing sufficiently high levels of acidity—HCl, pH less than 6.0 to 7.0, or other irritant substances (eg, osmotically active particles derived from refluxate or meals)—can potentially trigger a nociceptor response that is perceived as heartburn [30,31].

Conclusions

In summary, data suggest that the final common pathway for GERD-induced tissue injury is the failure of the IJC in the tissue defense system, leading to paracellular leaks in the esophageal epithelium and consequently DIS. This can be caused by increased contact time with acidic and nonacidic components of the refluxate and by substances in meals. Regardless of the cause, once the paracellular pathway is opened, as evidenced by dilatation of intercellular spaces, even modest levels of luminal acidity are capable of triggering the symptom of heartburn. Alternatively, other triggers for heartburn may be present, such as a hypertonic environment. If epithelial repair, either restitution or regeneration, exceeds cell necrosis, then the patient has NERD; if cell necrosis exceeds restitution and regeneration, then the patient has erosive esophagitis. Acid suppression with PPIs relieves heartburn and resolves DIS in most patients with erosive esophagitis and in many with NERD. If those with PPI-resistant NERD also have DIS, it would indicate that damage to the IJC is either initiated or perpetuated by other noxious components in the refluxate or meals. Much more work needs to be done, however, to establish the validity of this latter concept.

Disclosure

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References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Orlando RC: **Reflux esophagitis.** In *Textbook of Gastroenterology*. Edited by Yamada T, Alpers D, Owyang C, et al.: Philadelphia: Lippincott Williams & Wilkins; 1995:1214–1242.
2. Orlando RC: **Gastroesophageal reflux disease: Offensive factors and tissue resistance.** In *Gastroesophageal Reflux Disease*. Edited by Orlando RC. New York: Marcel Dekker; 2000:165–192.
3. Bernstein LM, Baker LA: **A clinical test for esophagitis.** *Gastroenterology* 1958, **34:**760–781.
4. Dean BB, Gano AD Jr, Knight K, et al.: **Effectiveness of proton pump inhibitors in nonerosive reflux disease.** *Clin Gastroenterol Hepatol* 2004, **2:**656–664.
5. Pehlivanov N, Liu J, Mittal RK: **Sustained esophageal contraction: a motor correlate of heartburn symptom.** *Am J Physiol Gastrointest Liver Physiol* 2001, **281:**G743–G751.
6. Fass R, Gasiorowska A: **Refractory GERD: what is it?** *Curr Gastroenterol Rep* 2008, **10:**252–257.
7. Tobey NA, Carson JL, Alkiek RA, Orlando RC: **Dilated intercellular spaces: a morphological feature of acid reflux-damaged human esophageal epithelium.** *Gastroenterology* 1996, **111:**1200–1205.
8. Solcia E, Villani L, Luinetti O, et al.: **Altered intercellular glycoconjugates and dilated intercellular spaces of esophageal epithelium in reflux disease.** *Virchows Arch* 2000, **436:**207–216.
9. Niessen CM: **Tight junctions/adherens junctions: basic structure and function.** *J Invest Dermatol* 2007, **127:**2525–2532.

10. Garrod D, Chidgey M: **Desmosome structure, composition and function.** *Biochim Biophys Acta* 2008, **1778:**572–587.
11. Tobey NA, Hosseini SS, Argote CM, et al.: **Dilated intercellular spaces and shunt permeability in nonerosive acid-damaged esophageal epithelium.** *Am J Gastroenterol* 2004, **99:**13–22.
12. Pope CI: **Gastroesophageal reflux disease (reflux esophagitis).** In *Gastrointestinal Disease: Pathophysiology, Diagnosis, Management,* edn 2. Sleisenger M, Fordtran J, eds. Philadelphia: WB Saunders; 1978:541–568.
13. Hopwood D, Milne G, Logan KR: **Electron microscopic changes in human oesophageal epithelium in oesophagitis.** *J Pathol* 1979, **129:**161–167.
14. Caviglia R, Ribolsi M, Maggiano N, et al.: **Dilated intercellular spaces of esophageal epithelium in nonerosive reflux disease patients with physiological esophageal acid exposure.** *Am J Gastroenterol* 2005, **100:**543–548.
15. Calabrese C, Bortolotti M, Fabbri A, et al.: **Reversibility of GERD ultrastructural alterations and relief of symptoms after omeprazole treatment.** *Am J Gastroenterol* 2005, **100:**537–542.
16. Bove M, Vieth M, Dombrowski F, et al.: **Acid challenge to the human esophageal mucosa: effects on epithelial architecture in health and disease.** *Dig Dis Sci* 2005, **50:**1488–1496.
17. Kahrilas PJ: **Dilated intercellular spaces: extending the reach of the endoscope.** *Am J Gastroenterol* 2005, **100:**549–550.
18. Carney CN, Orlando RC, Powell DW, Dotson MM: **Morphologic alterations in early acid-induced epithelial injury of the rabbit esophagus.** *Lab Invest* 1981, **45:**198–208.
19. Orlando RC, Powell DW, Carney CN: **Pathophysiology of acute acid injury in rabbit esophageal epithelium.** *J Clin Invest* 1981, **68:**286–293.
20.•• Tobey NA, Gambling TM, Vanegas XC, et al.: **Physicochemical basis for dilated intercellular spaces in non-erosive acid-damaged rabbit esophageal epithelium.** *Dis Esophagus* 2008, **21:**757–764.
This article establishes in a rabbit model the mechanism for the development of DIS in acid-exposed and injured esophageal epithelium.
21. Robles-Chillida EM, Rodrigo J, Mayo I, et al.: **Ultrastructure of free-ending nerve fibres in oesophageal epithelium.** *J Anat* 1981, **133:**227–233.
22. Rodrigo J, Hernandez CJ, Vidal MA, Pedrosa JA: **Vegetative innervation of the esophagus. III. Intraepithelial endings.** *Acta Anat (Basel)* 1975, **92:**242–258.
23. Tobey NA, Orlando RC: **Mechanisms of acid injury to rabbit esophageal epithelium. Role of basolateral cell membrane acidification.** *Gastroenterology* 1991, **101:**1220–1228.
24. Tobey NA, Reddy SP, Keku TO, et al.: **Mechanisms of HCl-induced lowering of intracellular pH in rabbit esophageal epithelial cells.** *Gastroenterology* 1993, **105:**1035–1044.
25. Sifrim D: **Acid, weakly acidic and non-acid gastro-oesophageal reflux: differences, prevalence and clinical relevance.** *Eur J Gastroenterol Hepatol* 2004, **16:**823–830.
26. Bor S, Caymaz-Bor C, Tobey NA, et al.: **Effect of ethanol on the structure and function of rabbit esophageal epithelium.** *Am J Physiol* 1998, **274:**G819–G826.
27. Long JD, Marten E, Tobey NA, Orlando RC: **Effects of luminal hypertonicity on rabbit esophageal epithelium.** *Am J Physiol* 1997, **273:**G647–G654.
28.• Farre R, van Malenstein H, De Vos R, et al.: **Short exposure of oesophageal mucosa to bile acids, both in acidic and weakly acidic conditions, can impair mucosal integrity and provoke dilated intercellular spaces.** *Gut* 2008, **57:**1366–1374.
This article documents in a rat model that DIS can be caused by physiologic stress.
29. Farre R, De Vos R, Geboes K, et al.: **Critical role of stress in increased oesophageal mucosa permeability and dilated intercellular spaces.** *Gut* 2007, **56:**1191–1197.
30. Bielefeldt K, Davis BM: **Differential effects of ASIC3 and TRPV1 deletion on gastroesophageal sensation in mice.** *Am J Physiol Gastrointest Liver Physiol* 2008, **294:** G130–G138.
31. Matthews PJ, Aziz Q, Facer P, et al.: **Increased capsaicin receptor TRPV1 nerve fibres in the inflamed human oesophagus.** *Eur J Gastroenterol Hepatol* 2004, **16:**897–902.