Relevance of Ultrastructural Alterations of Intercellular Junction Morphology in Inflamed Human Esophagus

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Background/Aims  
Detailed characterization of the ultrastructural morphology of intercellular space in gastroesophageal reflux disease has not been fully studied. We aimed to investigate whether subtle alteration in intercellular space structure and tight junction proteins might differ among patients with gastroesophageal reflux disease.

Methods  
Esophageal biopsies at 5 cm above the gastroesophageal junction were obtained from 6 asymptomatic controls, 10 patients with reflux symptoms but without erosions, and 18 patients with erosions. The biopsies were morphologically evaluated by transmission electron microscopy, and by using immunohistochemistry for tight junction proteins (claudin-1 and claudin-2 proteins).

Results  
The expressions of tight junction proteins did not differ between asymptomatic controls and gastroesophageal reflux disease patients. In patients with gastroesophageal reflux disease, altered desmosomal junction morphology was only found in upper stratified squamous epithelium. Dilated intercellular space occurred only in upper stratified squamous epithelium and in patients with erosive esophagitis.

Conclusions  
This study suggests that dilated intercellular space may not be uniformly present inside the esophageal mucosa and predominantly is located in upper squamous epithelium. Presence of desmosomal junction alterations is associated with increased severity of gastroesophageal reflux disease. Besides dilated intercellular space, subtle changes in ultrastructural morphology of intercellular space allow better identification of inflamed esophageal mucosa relevant to acid reflux.  

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Key Words  
Extracellular space; Gastroesophageal reflux; Microscopy, Electron, Transmission; Tight junctions

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Introduction

Gastroesophageal reflux disease (GERD), with typical presentation of heartburn and acid regurgitation, is a common disorder with complex definitions in the 21st century. Its atypical presentation includes asthma, chronic cough, or chronic laryngitis. It can be further categorized based on esophagogastroduodenoscopy (EGD) into erosive esophagitis (EE) and non-erosive reflux disease (NERD), in which the prevalence of the latter group was reported to be around 50%.

Overt reflux associated with esophageal injury occurs when there is a failure in the esophageal defense mechanisms, which may include limiting reflux frequency, reducing acid contact time between epithelium and acid, and tissue resistance against injury by acid. Of these defense mechanisms, tissue resistance, mainly at tissue level, consists of a mucus- and bicarbonate-containing unstirred water layer, the apical membrane of esophageal mucosa, the intercellular junctional complexes, bicarbonate buffering, and H+ ion transporters located on the basolateral membrane. The intercellular junctional complexes allow cells to adhere more tightly to each other by bridging protein connections mediated by claudins and occludins for the tight junction, e-cadherin for the adherens junction, and desmocollin and desmoglein for the desmosomes.

The claudins constitute a family of 24 distinct transmembrane proteins that are composed of 4 transmembrane domains and two extracellular loops, which are involved in the homophilic and heterophilic interactions with other adjacent claudins. Claudins display distinct patterns of expression that are tissue-specific and account for much of the diversity in trans-epithelial permeability of the tight junction, and such differences also lead to varying degrees of vulnerability of the tight junction to diseases. Alterations in tight-junction proteins (TJP) may increase the permeability of the esophageal mucosa, therefore impairing tissue resistance of esophageal epithelium. It is yet unclear whether the expression of these proteins is altered in reflux esophagitis in humans.

The dilatation of intercellular space (ICS) has been suggested to be a pathological marker for acid related esophageal damage based on studies in rabbit esophageal mucosa and human esophageal mucosa. This subtle change is poorly identified on light microscopy (LM), but easily visualized by transmission electron microscopy (TEM). Subsequently, it is observed in association with bile reflux, NERD, reversibility after GERD therapy. However, a recent study did not find dilated ICS in patients with GERD and chronic laryngitis, posing a question whether dilated ICS occurs uniformly in patients with GERD. More recently, ICS was demonstrated to vary among subdivided layers of esophageal mucosa, suggesting the need for reconsideration of studying TEM by individual layer of esophageal mucosa, although earlier studies did not perform TEM analysis of ICS in such manner.

According to these considerations, the aims of this study were to characterize intercellular junction alterations and ICS of esophageal mucosa as measured by TEM and to investigate TJP of esophageal epithelium in patients with GERD.

Materials and Methods

Subjects

The study protocol was approved by Research Ethics Committee of Tzu Chi Medical Center. Each subject agreeing to participate provided informed consent. GERD patients with heartburn and/or acid regurgitation of at least 6 months’ duration and history of response to proton pump inhibitor (PPI) were enrolled in the study. Asymptomatic controls were selected on the basis of absence of reflux symptoms and inflammation in the distal esophagus and undergoing EGD for other clinical reasons or healthy screening. Asymptomatic controls had no history of reflux disease or esophageal disease, and showed a grossly normal esophagus on endoscopy. Patients were excluded if they had the following conditions: (1) Barrett’s esophagus, (2) esophageal strictures, (3) previous gastrointestinal surgery, and (4) concurrent use of PPI and/or other antisecretory agents. All subjects withheld acid suppressive therapy such as PPI and histamine type 2 receptor antagonists 4 weeks before EGD evaluation.

Endoscopic Evaluation

We evaluated the distal portion of the esophagus during standard EGD to determine the presence of mucosal injury. The extent of mucosal damage was assessed using the Los Angeles grading system. EE was defined by the presence of endoscopically detectable mucosal breaks (erosions or ulcer), while the diagnosis of NERD was based on no endoscopically detectable mucosal lesions such as erosions or ulcers. Patients with EE were further subclassified into mild EE if their EGD showed grade A esophagitis, and moderate EE if EGD showed at least grade B.

During the EGD, esophageal biopsies were obtained at 5 cm