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Role of Saliva in Esophageal Defense: Implications in Patients With Nonerosive Reflux Disease

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Abstract: *Background:* It has been previously demonstrated that patients with reflux esophagitis exhibit a significant impairment in the secretion of salivary protective components versus controls. However, the secretion of salivary protective factors in patients with nonerosive reflux disease (NERD) is not explored. The authors therefore studied the secretion of salivary volume, pH, bicarbonate, nonbicarbonate glycoconjugate, protein, epidermal growth factor (EGF), transforming growth factor alpha (TGF-α) and prostaglandin E2 in patients with NERD and compared with the corresponding values in controls (CTRL). *Methods:* Salivary secretion was collected during basal condition, mastication and intraesophageal mechanical (tubing, balloon) and chemical (initial saline, acid, acid/pepsin, final saline) stimulations, respectively, mimicking the natural gastroesophageal reflux. *Results:* Salivary volume, protein and TGF-α outputs in patients with NERD were significantly higher than CTRL during intraesophageal mechanical ($P < 0.05$) and chemical stimulations ($P < 0.05$). Salivary bicarbonate was significantly higher in NERD than CTRL group during intraesophageal stimulation with both acid/pepsin ($P < 0.05$) and saline ($P < 0.01$). Salivary glycoconjugate secretion was significantly higher in the NERD group than the CTRL group during chewing ($P < 0.05$), mechanical ($P < 0.05$) and chemical stimulation ($P < 0.01$). Salivary EGF secretion was higher in patients with NERD during mechanical stimulation ($P < 0.05$). *Conclusions:* Patients with NERD demonstrated a significantly stronger salivary secretory response in terms of volume, bicarbonate, glycoconjugate, protein, EGF and TGF-α than asymptomatic controls. This enhanced salivary esophagoprotection is potentially mediating resistance to the development of endoscopic mucosal changes by gastroesophageal reflux.

Key Indexing Terms: Nonerosive reflux disease; Salivary protection; Bicarbonate; Glycoconjugate; Epidermal growth factor. **[Am J Med Sci 2015;349(5):385–391.]**

Gastroesophageal reflux disease (GERD) is a highly prevalent disease in the western world and affects approximately up to 20% of adults and nearly 25 million experience heartburn on a daily basis.[1–3] Heartburn is elicited by the contact of the esophageal mucosal chemoreceptors with aggressive factors, predominantly acid, pepsin and bile components on the luminal perimeter of the esophageal mucosa during the episodes of gastroesophageal reflux.[4] Response within chemoreceptors is subsequently conveyed through the afferent autonomic fibers of the esophagosalivary reflex pathway, resulting in its modulatory impact on the secretory function of salivary glands.[5,6] Salivary secretion of water and inorganic components (electrolytes and buffers) is mediated predominantly by parasympathetic pathways whereas secretion of organic components (proteins, glycoconjugates and peptides) by sympathetic pathways.[7–10]

Salivary secretion combined with a local secretory response within the esophageal submucosal mucous glands defines the quality and the quantity of the esophageal preepithelial barrier, which is pivotal in the maintenance of the integrity of the esophageal epithelium.[10–14] Therefore, heartburn, although often worrisome for the patient, is a beneficial symptom if it is capable of inducing an adequate salivary secretory response facilitating neutralization and inactivation of the aggressive factors within the esophageal lumen and thus restoring near-neutral pH within the esophageal lumen.[12,15,16]

The majority of patients with GERD (up to 60%) have no visible erosive abnormalities during standard endoscopic examination, and this subgroup is defined as nonerosive reflux disease (NERD).[17,18] NERD is a condition in which typical reflux symptoms, heartburn and regurgitation are defined as troublesome in patients with negative endoscopy. The absence of visible lesions on endoscopy and the presence of troublesome reflux-associated (acidic, weakly acidic or non-acid reflux) symptoms are the 2 key factors for the definition of NERD. This clinical entity also requires abnormal impedance-pH monitoring for its diagnosis.[19] It has been demonstrated previously by Rourk that patients with reflux esophagitis (RE) fail to illicit a vigorous secretory response of salivary epidermal growth factor (EGF) during intraesophageal mechanical and chemical stimulations.[20] The amount of secretion of salivary protective factors in patients with NERD remains unknown. It is legitimate to surmise that a vigorous and protective salivary secretory response in terms of its major protective factors to an aggressive intraesophageal challenge in patients with NERD may prevent endoscopic mucosal injury.

MATERIALS AND METHODS

Subjects

The study was approved by the Human Subject Committee and conducted on 33 asymptomatic volunteers (15 women and 18 men; mean age of 39 years; range, 26–56 years) and 10 white patients (4 women and 6 men; mean age of 40 years; range, 27–64 years) with a history of GERD (heartburn as a predominant symptom) confirmed by 24-hour pH monitoring and grossly normal endoscopy. Informed

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consent was obtained from all subjects. All subjects were not afflicted with any acute illness, did not use tobacco, alcohol or chewing gum, did not receive any medications including acid suppressive therapies and antisecretory medications 14 days before the procedure, and never had any dysfunction of mastication.

Salivary Sample Collection

Subjects expectorated all saliva collected in their mouth every 10 seconds and were instructed not to swallow during the procedure. The salivary samples were sequentially collected on ice during the same time of the day for each subject as follows: (1) basal saliva during the first 10 minutes, (2) saliva produced during stimulation by parafilm chewing (mastication) during the following 5 minutes, (3) saliva produced by tubing following the placement of the intraesophageal catheter during 2 consecutive 1.5-minute intervals, (4) saliva produced following inflation of both intraesophageal balloons during 2 consecutive 1.5-minute intervals and (5) saliva produced during the esophageal perfusion with initial saline (NaCl), hydrochloric acid (HCl), HCl/pepsin and final saline consecutively, 4 samples each totaling 16 consecutive 1.5-minute intervals. The order of perfusions is very important as we go from initial saline, which represents "physiological" neutral pH reflux, followed by HCl where hydrogen ions start diffusing into the mucous barrier quickly initiating response, followed by HCl/pepsin that erodes the mucous barrier injuring the surface epithelium and finally saline, which calms down the reflux episode.

Esophageal Perfusion Catheter

Esophageal perfusion was performed with a specially designed 6-channeled catheter manufactured by Wilson-Cook Company (Chapel Hill, NC), as described in detail by Sarosiek et al.[21] Four larger diameter channels were used for infusion and aspiration of the perfusate, gastric juice and incidentally swallowed saliva, which is retained above the upper balloon. Two smaller diameter channels were used for inflation of the upper and lower balloons to compartmentalize the segment of the lower esophagus.[7,8,10,20–24]

Perfusing Solutions

Esophageal perfusion in all subjects was performed using fresh 10 mL solutions for each 1.5-minute interval: (1) NaCl (0.15 M) that corresponds to 0.9% saline; (2) HCl (0.01 M; pH 2.1), this concentration and pH of HCl was chosen to closely resemble the content of gastroesophageal refluxate[25,26]; and (3) HCl (0.01 M; pH 2.1) with pepsin, where pepsin (0.5 mg/mL; Sigma Chemical Co., St. Louis, MO) was dissolved in the concentration that corresponds to the average proteolytic activity of human gastric juice.[27,28]

Esophageal Perfusion Procedure

Subjects were placed in the semirecumbent left-sided position. The nasopharynx was anaesthetized with xylocaine gel, the esophageal catheter was inserted into the esophagus through the nares and the balloons of the catheter were gently insufflated to seal the esophageal lumen. This procedure allows the compartmentalization of 3.75-cm segment of the esophagus between the balloons.[14,21,29,30] During each perfusion period of 1.5-minute interval, the entire 10 mL solution of perfusate was circulated within the isolated segment of esophagus for a total duration of 24 minutes for each subject. The final value of each perfusion represents the mean value of 4 consecutive 1.5-minute intervals of perfusions or recirculations.

Analysis of Salivary Secretory Components

Salivary volume was assessed using a sialometer (Proflow Incorporated, Amityville, NY).[8,20,22] Salivary pH was monitored using the Expandable Ion Analyzer EA 940 (Orion Res., Boston, MA).

The salivary bicarbonate and nonbicarbonate buffers were analyzed by titration and back-titration methodology using TitraLab 90 (Radiometer America Inc., Chicago, IL).[31] Secretions form a thin film on the mucosa and allows the evolution of CO_2 formed from acid-base interactions. Therefore, the esophageal bicarbonate buffer value would be equilibrated with CO_2 tension of the lumen.[31,32] This was the rationale for choosing titration to pH of 4.0 for the assessment of esophageal bicarbonate in an open system (without covering with a layer of liquid paraffin oil) with continuous CO_2-free bubbling. The bicarbonate concentration was calculated using the difference in the amount of acid initially required to titrate the sample from its starting pH to pH 4.0 and the amount of base required to back-titrate the sample to its original pH after development of the CO_2. The difference between the back-titration from pH 4.0 to its original starting value and the similar run of the buffer-free blank solution was used to calculate nonbicarbonate buffers.[31,32] In addition, this methodology was always validated by the titration of known concentrations of bicarbonate and nonbicarbonate in the standard solutions.

Salivary glycoconjugate (predominantly mucin) was measured using the periodic–acid Schiff methodology.[14,29,31] Salivary EGF was assessed by radioimmunoassay (RIA) using a commercially available kit (Amersham, Arlington Heights, IL).[8,20,21,31]

Salivary transforming growth factor alpha (TGF-α) was recorded using a commercially available RIA kit based on highly specific sheep anti-human TGF-α antibodies (Biomedical Technologies Inc., Stoughton, MA).[31,33] The separation between bound and unbound TGF-α was performed using donkey anti-sheep IgG and polyethylene glycol. Human recombinant TGF-α (BTI) was used for a standard curve. All samples were centrifuged at 4°C and 3,000 rpm for 20 minutes, which are the conditions required to spin down cellular debris, plasma membrane sheets and nuclei.

Salivary prostaglandin E_2 (PGE_2) was measured using an RIA kit (Amersham).[30] This RIA method is based on highly specific antibodies directed to oximated form of PGE_2. Salivary protein was monitored by the Lowry methodology.[14]

Data Processing and Statistical Analysis

Data were measured as mean values of salivary collections at basal level, during parafilm chewing, following placement of tubing, following inflation of balloons and during the perfusion intervals. All results were expressed as mean ± SEM. Statistical analysis by analysis of variance was performed using Σ-Stat software (Jandel Scientific, San Rafael, CA).

RESULTS

Salivary Inorganic Protective Components

Salivary volume in patients with NERD was significantly higher than control group (CTRL) during mechanical stimulation with balloons (4.67 ± 1.16 mL/min versus 3.16 ± 0.32 mL/min, $P < 0.05$) and chemical stimulation with HCl/pepsin and final saline (4.12 ± 0.38 mL/min versus 2.83 ± 0.33 mL/min, $P < 0.05$ and 4.39 ± 0.54 mL/min versus 2.75 ± 0.33 mL/min, $P < 0.05$, respectively), as shown in Figure 1. The