Role of salivary mucin in the protection of rat esophageal mucosa from acid and pepsin-induced injury

MINE KINOSHITA, EISUKE KUME, SHIGEKI Igarashi, Nobuko Saito, and HIROSHI Narita

Tanabe Serylaku Company, Ltd., Discovery Research Laboratory, Toda, Saitama 335-8505, Japan

Kinoshita, Mine, Eisuke Kume, Shigeki Igarashi, Nobuko Saito, and Hiroshi Narita. Role of salivary mucin in the protection of rat esophageal mucosa from acid and pepsin-induced injury. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G796–G800, 1999.—The mucosal defensive mechanisms of the esophagus against acid and pepsin remain to be elucidated. In the present study, we investigated the contribution of the salivary mucin in maintaining the integrity of the esophageal mucosa. When an everted esophageal sac, isolated from normal rat, was treated with N-acetyl-L-cysteine, a mucolytic agent, the amount of glycoprotein in the gel layer adherent to the epithelium was completely depleted and the susceptibility of the mucosa against acidified pepsin-induced digestion increased. In sialoadenectomized rats, 7 days after extirpation, the amount of glycoprotein adherent to the esophageal epithelium was definitely reduced, and the esophageal mucosa was significantly vulnerable to acidified pepsin-induced digestion compared with the sham-operated rats. Induction of regurgitation of the gastric juices into the esophagus resulted in the development of severe hemorrhagic esophageal lesions only in the sialoadenectomized rats but not in the sham-operated rats. In conclusion, the glycoprotein in the adherent gel layer in rat esophagus, which mainly derives from salivary glands, plays an important role in the preepithelial defense to maintain the integrity of the esophageal mucosa against acid and pepsin.

reflux esophagitis; salivary glands; mucus glycoprotein; epidermal growth factor; sialoadenectomy

THE GASTRODUODENAL epithelium maintains its integrity under the attack of acid, pepsin, and ulcerogenic agents through defensive mechanisms, such as mucus and bicarbonate secretion and mucosal blood flow. The esophageal epithelium is also exposed to acid, pepsin, and noxious agents; however, the mechanisms of how the esophageal epithelium withstands these harmful agents have not been as well documented. Recent clinical studies suggested the involvement of salivary and esophageal submucosal glands in the esophageal defense system, since the secretion of alkaline, mucins, epidermal growth factor (EGF), and PGE2 from these glands was enhanced by esophageal luminal perfusion with acid and/or pepsin in healthy subjects (3, 11, 16). Furthermore, the salivary and esophageal secretion in response to esophageal chemical stimulation has been reported to be impaired in patients with reflux esophagitis (10, 15). There have been, however, few experimental data to confirm the protective role of these components from esophageal and salivary glands.

In the gastroduodenal tracts, the elastic mucus gel layer covering the epithelium serves as an unstirred buffering barrier, with epithelium-secreted bicarbonate protecting against acid and a diffusion barrier for luminal pepsin (1). Thus, in the esophagus, mucin, which originates from salivary and esophageal submucosal glands, may play an important role in the mucosal protection as a preepithelial barrier. In the present study, we first estimated the role of the adherent mucin in the protection of the esophagus against acid and pepsin by in vitro studies using N-acetyl-L-cysteine (NAC), a mucolytic agent. Second, to elucidate the contribution of the salivary mucin in the defense mechanisms of esophageal mucosa, we assessed the effects of sialoadenectomy on both the esophageal adherent mucin and the mucosal resistance against attack by gastric juices.

MATERIALS AND METHODS

Animals and Drugs

Male Sprague-Dawley rats (Charles River Japan, Kanagawa, Japan) weighing 160–230 g were used.

NAC and Alcian blue 8GX were purchased from Nacalai Tesque (Kyoto, Japan), EGF extracted from mouse submaxillary gland was obtained from Toyobo (Osaka, Japan), and purified porcine pepsin (P6887, 3400 U/mg protein) was purchased from Sigma Chemical (St. Louis, MO).

Effect of NAC

Under ether anesthesia, a 5-cm length of esophagus was excised and immediately everted, and both ends of the esophagus were ligated. This everted sac was incubated in a 0.2% NaCl solution with or without 1% NAC, a mucolytic agent, for 20 min at room temperature. After the incubation, the following three experiments were performed.

Experiment 1. After incubation with NAC, the everted esophagus was placed in ice-cold Carnoy’s solution for 2 h and subsequently in absolute ethanol (9). The circular ring strip of the esophagus was embedded in paraffin. Sections (4 µm) were stained with periodic acid-Schiff and examined under a light microscope.

Experiment 2. After incubation with NAC, the esophageal sac was immediately placed in 3 ml of ice-cold distilled water, and the adherent materials to the esophageal surface were gently scraped with a spatula. The scraped samples from six sacs were pooled and lyophilized. Mucus glycoprotein was isolated from the lyophilized sample by gel filtration and measured according to the method of Ohara et al. (12). The sample was dissolved by homogenization in 3 ml of 0.05 M Tris·HCl buffer (pH 7.2), Triton X-100 (100 µl) was added, and the sample was incubated for 1 h at 37°C. After centrifugation (8,000 g, 4°C, 30 min), the supernatant was collected. Two milliliters from the collected supernatant were applied to the gel in tubes of 0.2% NaCl solution with or without 1% NAC.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

G796
Experiment 2. One week after the sialoadenectomy, under halothane anesthesia, the submandibular-sublingual salivary gland complexes were removed bilaterally after their ducts were ligated (17). Sham-operated rats served as controls. One week after the sialoadenectomy, the integrity of the esophageal mucosa was assessed with the following protocols.

Experiment 1. One week after the sialoadenectomy, under ether anesthesia, a 5-cm length of esophagus was excised. Mucus glycoprotein in the materials adherent to the epithelium was isolated and measured as mentioned above.

Experiment 2. One week after the sialoadenectomy, under ether anesthesia, a 5-cm length of esophagus was excised and immediately everted, and both ends of the esophagus were ligated. The everted sac was incubated in 0.1 N HCl-0.2% NaCl solution containing porcine pepsin (1 mg/ml) for 30 min at 37°C. Subsequently, the sac was removed, and one volume of 7% TCA was added to the incubation solution. After centrifugation (1,500 g, 4°C, 10 min), the TCA-soluble peptide in the supernatant was determined by the method of Folin and Ciocalteau (5) using tyrosine as a standard.

Experiment 3. Six days after the sialoadenectomy, the rats were fasted for 24 h with free access to water. After the fasting, under ether anesthesia, the pylorus and limiting ridge (transitional region between forestomach and glandular portion) were ligated to induce the reflux of gastric contents into the esophagus (13); 2.5 h later, the esophagus was excised and fixed with 10% neutral formalin solution, and the circular ring strip was embedded in paraffin. Sections (4 µm) were stained with hematoxylin and eosin and examined under a light microscope.

Effect of Sialoadenectomy

Under halothane anesthesia, the submandibular-sublingual salivary gland complexes were removed bilaterally after their ducts were ligated (17). Sham-operated rats served as controls. One week after the sialoadenectomy, the integrity of the esophageal mucosa was assessed with the following protocols.

Table 1. Effects of NAC on the amount of adherent glycoprotein and the vulnerability to acidified pepsin-induced digestion in the isolated rat esophagus

<table>
<thead>
<tr>
<th>Glycoprotein, µg hexose/g wet tissue</th>
<th>TCA-soluble Peptide, µg tyrosine/g wet tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.8 ± 7.3</td>
</tr>
<tr>
<td>NAC</td>
<td>27.5 ± 8.3</td>
</tr>
</tbody>
</table>

Values are means ± SE for 4 and 6 preparations in the glycoprotein determination and in the pepsin-induced digestion, respectively. NAC, N-acetyl-L-cysteine; ND, not detectable. *P < 0.01 compared with control.
The esophageal mucosa was significantly reduced (Fig. 2, top), whereas the epithelium remained histologically intact without any degenerative changes (data not shown). The esophageal mucosa, isolated from sialoadenectomized rats, was more vulnerable to acidified pepsin-induced digestion compared with that from the sham-operated rats (Fig. 2, bottom).

The induction of regurgitation of the gastric contents into the esophagus did not affect the microscopic appearance of the esophageal mucosa in the sham-operated rats 2.5 h after the operation (Fig. 3A). In contrast, the reflux of the gastric juice disrupted the esophageal epithelium and vast severe hemorrhagic lesions developed that reached to the muscle layer in one-half of the sialoadenectomized rats (Fig. 3B). To assess the effect of removal of salivary glands on gastric secretory activity, the acid concentration and the pepsin activity were measured. The sialoadenectomy did not affect the acid concentration but increased the pepsin activity in gastric juices (Fig. 4). The EGF treatment did not prevent the sialoadenectomy-induced increase in vulnerability of the esophagus against the gastric juices; nevertheless, it suppressed the sialoadenectomy-induced increase in pepsin activity (Fig. 4).

DISCUSSION

The gastrointestinal tract is covered by a viscoelastic and lubricant layer of highly glycosylated proteins, termed mucins. This adherent mucus gel layer protects the gastrointestinal epithelium from acid, pepsin, nox-
ious agents, and microorganisms (1). In the present study, the elastic materials adherent to the surface of the rat esophageal epithelium contained the high-molecular-weight glycoprotein. Mucolytic treatment with NAC depleted this adherent glycoprotein without any microscopic damage, resulting in a marked increase in the susceptibility of the esophageal epithelium to acid- and pepsin-induced digestion. These findings indicate that the rat esophageal mucosa is covered with mucus glycoprotein, and this adherent mucus layer serves to buffer against the attack of acid and pepsin in the esophagus as well as in the stomach. Therefore, other damages, such as impaired mucosal resistance, are possibly involved in the pathogenic mechanisms of reflux esophagitis. Some clinical reports suggest a contribution of organic (mucins and EGF) and inorganic secretions (alkaline) from salivary and esophageal submucosal glands to the preepithelial defense in esophagus. In healthy subjects, the secretion of alkaline, mucins, EGF, and PGE2 from these glands was enhanced by esophageal luminal perfusion with acid and/or pepsin (3, 11, 16). The salivary and esophageal secretion in response to esophageal chemical stimulation was impaired in patients with reflux esophagitis (10, 15). These reports are not able to clarify the role of each component of the salivary and esophageal glands in the esophageal defense, despite indication of a comprehensive participation of these components in the maintenance of esophageal integrity. In the present study, we clearly demonstrated that salivary-derived mucin covered the rat esophageal epithelium, and the depletion of the adherent mucus layer worsened the acid- and pepsin-induced esophageal damage. In conclusion, the adherent mucus derived from salivary glands withstands the deleterious impact of the reflux of gastric contents as a preepithelial barrier.

We thank S. Kurabe, E. Ohtsuka, and T. Yahagi for skillful technical assistance in the histological study. Address for reprint requests and other correspondence: M. Kinoshita, Tanabe Seiyaku, Ltd., Discovery Research Laboratory, Dept. of Pharmacology, 2-2-50, Kawagishi, Toda, Saitama, 335-8505, Japan (E-mail: mine-k@tanabe.co.jp).

Received 11 January 1999; accepted in final form 7 July 1999.

REFERENCES