Effects of Caustic Lye Injury to the Esophageal Smooth Muscle Reactivity: In Vitro Study

Melih Tugay, M.D., *,1 Tijen Utkan, Ph.D., † and Zafer Utkan, M.D.‡

*Department of Pediatric Surgery, †Department of Pharmacology and Experimental Medical Research Center, and ‡Department of General Surgery, Kocaeli University Medical Faculty, Kocaeli, Turkey

Submitted for publication February 5, 2003

Purpose. We investigated late effects of caustic lye injury on esophageal smooth muscle reactivity in the rat model.

Materials and methods. Male Sprague–Dawley rats were divided into two groups. Through a median laparatomy incision, abdominal esophageal segment was isolated. Orogastric and gastric (via gastrotomy) catheters were placed and tied over the isolated esophageal segment. Saline (0.9%) or 50% sodium hydroxide (1 ml) solution were instilled via orogastric catheter to the isolated segment in the control and caustic esophagus (CE group) groups, respectively. Then, the esophagus was rinsed with 0.9% saline via gastric catheter. The esophagus was removed and studied in organ chambers 28 days after the operation.

Results. Carbachol- and KCl-induced contractile responses of esophageal smooth muscle were significantly reduced in the CE group with decreased \( E_{\text{max}} \) value compared with the control group. Relaxant responses to serotonin were significantly reduced in the CE group with decreased \( E_{\text{max}} \) value compared with the control group. No significant differences were found in \( E_{\text{max}} \) and \( pD_2 \) values for papaverine acting on esophageal strips from the two groups.

Conclusion. The results provide evidence that a surgically created caustic injury causes impaired smooth muscle reactivity that may contribute to esophageal motor dysfunction.© 2003 Elsevier Inc. All rights reserved.

Key Words: esophagus; caustic injury; in vitro; rat.

INTRODUCTION

Caustic esophageal injury is a common health problem in the pediatric population with late effects [1]. Children usually suffer from dysphagia or gastroesophageal reflux (GER)-related symptoms because of caustic esophageal injury resulting in the failure of conventional therapy [2–5]. Important esophageal motor function changes have been reported based on the pH monitoring, manometric, radiological, and scintigraphic studies in such patients [3–7]. Until now, low-amplitude nonperistaltic contractions, and narrowing and shortening of the esophagus have been considered for impaired esophageal motor function [1–7]. In this background, our aim was to further evaluate the mechanism of impaired motor function after caustic injury by using organ chambers in the rat model.

MATERIALS AND METHODS

Experiments were performed on male Sprague–Dawley rats (weighing 195–225 g) obtained from the Experimental Medical Research Unit (DETAB, Kocaeli University Medical Faculty, Kocaeli). They were kept in an individual solid bottom plastic cages on sawdust bedding at a temperature- and humidity-controlled room (22 ± 30°C and 62% ± 7%, respectively) in which a 12-h/12-h light/dark cycle was maintained (08:00–20:00 h light). Rats were deprived of food but allowed free access to water for 8 h before and 24 h after surgery.

Surgical Procedure

The model described by Gehanno and its modification by Liu was used to create standard caustic esophageal injury [8, 9]. The animals anesthetized with a ketamine hydrochloride (20 mg/100 g body weight, i.p.). Almost 1.5-cm intraabdominal esophageal segments were isolated through a median laparatomy incision. Two catheters (1 mm internal diameter and 2.2 mm external diameter, Dow Corning, Midland, MI) via oral and gastric routes were placed in this segment. Both catheter tied and secured with 3-O silk sutures over the esophagus. One milliliter of 0.9% saline solution in the control group (\( n = 8 \)) and 1 ml of 50% sodium hydroxide solution in the caustic esophagus (CE group; \( n = 10 \)) were instilled to esophagus through oral catheter for 90 s. Then the esophagus was rinsed for 15–20 s with distilled water through the gastric catheter. After then
catheters were withdrawn, and gastric insertion site was repaired. All animals were harvested using overdose of ether 28 days after the operation. Esophagus was dissected through thoracoabdominal incision and 1.5 cm thoracic segment proximal to abdominal stricture formation was prepared for histological and organ bath studies as described previously [10].

Organ Chamber Experiments

The experiments have been made in the Experimental Medical Research Unit (DETAB, Kocaeli University Medical Faculty, Kocaeli). These experiments were conducted in accordance with the Declaration of Helsinki and the Kocaeli University Ethics Committee granted ethical approval. Intrathoracic esophageal segment above the injured intraabdominal esophagus in the CE group and esophageal segment from the same localization in the control group were studied in 20 ml water-jacketed organ baths for isometric tension recording. The strips were tied with silk thread to a force transducer (PDT 10-A, May JOBS 99, COMMAT Iletisim Co. Ankara, Turkey) for the measurement of isometric force, which was continuously displaced and recorded on an online computer via four-channel transducer data acquisition system (MP30B-CE, BIOPAC Systems Inc., Santa Barbara, CA) using software (BSL PRO v 3.6.7, BIOPAC Systems Inc., Santa Barbara, CA), which also had the capacity to analyze the data. The tissue baths contained Tyrode’s solution composed of (millimoles per liter): NaCl 136.0; KCl 2.7; CaCl₂ 1.8; MgCl₂ 1.05; NaH₂PO₄·H₂O 0.42; NaHCO₃ 11.9; glucose 5.5. The solution was gassed with 95% O₂ and 5% CO₂ during the study and the temperature was maintained at 37°C. After mounting, the strips were allowed to equilibrate under a resting tension 0.5 g for 90 min. During this period the bath fluid was routinely changed every 15 min. Agonists were added directly to the organ bath. At the completion of each experiment, tissues were lightly blotted and weighted.

In a series of preliminary experiments lower esophageal strips were stimulated with 80mmol/L KCl. In examining the contractile response to the muscarinic agonist carbachol (10⁻⁶–3.10⁻⁶ mol/l), cumulative concentration–response curve was constructed in a stepwise manner after the response to the previous concentration had reached a plateau. After completion of carbachol dose–response curve, tissues were washed for a further 60 min and precontracted with a submaximal concentration of carbachol (10⁻⁶–3.10⁻⁶ mol/l). After the contractions reached a plateau, concentration-response relationships for serotonin (10⁻⁶–10⁻⁷ mol/l) and papaverine (10⁻⁶–10⁻⁷ mol/l), was obtained in a cumulative manner.

Analysis of Data

Results are expressed as mean ± SEM where n equals the number of animals. The contractile force was expressed as milligrams of developed tension per milligram of tissue wet weight. The relaxant effects of agonists were expressed as a percentage of the precontracting to carbachol. Concentration-response curves were fitted by nonlinear regression with simplex algorithm and Eₘₐₓ (maximum response) and pD₂ (i.e., the negative logarithm of the concentration for the half maximal response; EC₅₀) values were calculated using the software of transducer data acquisition system.

Statistical Analysis

Results are expressed as mean ± SEM. Statistically significant differences between the two groups were calculated by Student’s t test. Probabilities of less than 5% (P < 0.05) considered significant.

Drugs

After chemicals were obtained from Sigma Chemical (St. Louis, MO): carbamylcholine chloride, serotonin creatinine sulfate and papaverine hydrochloride. In the high K⁺ solution NaCl was exchanged for equimolar amounts of KCl. Drugs were prepared daily in distilled water and kept in ice during the course of experiments.

RESULTS

Caustic injury was appeared in the form of stricture in the entire abdominal esophageal segment where the 50% NaOH was applied. Mild dilation above the uninjured entire esophageal segment was also noticed. All esophageal specimens from the CE group had clear findings of caustic injury, such as severe narrowing in the lumen and thickening in the wall in hematoxylin and eosin-stained specimens providing validity of the model [7, 8]. No lesion was observed in the esophagus of the control group.

The cumulative addition of carbachol (10⁻⁹–3.10⁻⁴ M) produced concentration-dependent contractions of the esophageal strips. The contractile response was significantly decreased in the strips obtained from the CE group compared with the control group (Fig. 1). There was a significant decrease in the maximum responses (Eₘₐₓ) but this change in maximum effect to carbachol occurred without a significant change in pD₂ value (Table 1). The contractile response elicited by KCl was significantly decreased in the CE group compared with the control group (Fig. 2, Table 1).

Serotonin produced concentration-dependent relaxation in submaximally precontracted (10⁻⁶–3.10⁻⁶ M carbachol) esophageal strips obtained from the two groups. Tension induced by carbachol was similar in the both groups. The relaxation elicited by serotonin was significantly decreased in the CE group with decreased Eₘₐₓ value compared with the control group but no significant change in the pD₂ value was obtained (Fig. 3, Table 1).

When strips were contracted with carbachol for the study of responses to papaverine similar tension was achieved in the groups. The relaxation elicited by papaverine was similar in the two groups and there was no significant change in the pD₂ or Eₘₐₓ values (Fig. 4, Table 1).

DISCUSSION

The ingestion of caustic substance is a frequent accident among children and can cause to severe damage to esophagus. Previous studies indicated that formation of esophageal stricture, impaired motility, and GER are very frequent sequelae of caustic esophageal injury and should be taken into account when evaluating symptoms and deciding on the therapeutic strategy to be followed [1–7].

In alkali ingestion, tissue penetration with liquefactive necrosis is followed by destruction of the epithelium and submucosa with intense inflammation. Depending on the degree of burn, inflammation may
extend through the muscle layer until perforation occurs. Eventually, impaired esophageal function may be end result of fibrotic stricture, shortened esophagus, and reduction in neuron number in the myenteric plexus [1, 4–6]. Therefore, mechanical factors and injury to the myenteric plexus may underlie in the alterations of the esophageal motility and GER after caustic injury. Esophageal shortening may lead to lower esophageal sphincter incompetence and esophageal stricture may result in esophageal rigidity after caustic injury. Then, esophageal motor dysfunction may result in GER after caustic esophageal injury [5, 6, 11]. Moreover, intensity of the esophageal stricture was not always well correlated with the impaired esophageal function [6, 7]. Therefore, impaired esophageal motility cannot be solely attributed to the stricture formation or esophageal shortening.

In the present study, esophageal smooth muscle was analyzed after caustic injury using in vitro technique. Both Impaired receptor-mediated (carbachol, serotonin) and nonreceptor-mediated (KCl) smooth muscle reactivity were demonstrated. Regardless of the mechanism of agonist action marked decreased smooth muscle reactivity was found in the CE group compared with the control group. Even though the rat esophagus differs from that of the human, our results seem to be valid. Therefore, impaired smooth muscle forces may underlie some of the abnormal esophageal motility patterns, such as delayed transit and low amplitude non-propulsive esophageal waves [6]. However, there are a few drawbacks in this study that must be considered when analyzing the results. First, 50% NaOH concentration was higher than the concentration caused to esophageal injury in children. However, this concentration caused a very clear injury that provided more accurate evaluation of the esophageal smooth muscle reactivity. Esophageal stricture caused by high concentration NaOH was also reason to partial esophageal obstruction very similar to human situation. Second, esophageal segment above the injured site was used because of the fibrotic segment did not respond to agonist studies in the preliminary study.

### TABLE 1

<table>
<thead>
<tr>
<th>Maximum Contraction Values (E_max mg/mg) for Carbachol and KCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Carbachol</td>
</tr>
<tr>
<td>Serotonin</td>
</tr>
<tr>
<td>Papaverine</td>
</tr>
<tr>
<td><strong>E_max</strong></td>
</tr>
<tr>
<td>KCl</td>
</tr>
<tr>
<td>Carbachol</td>
</tr>
<tr>
<td>Papaverine</td>
</tr>
</tbody>
</table>

Note. Values are arithmetic mean ± SEM. Maximum relaxation values (% of 10–6–3.10–6M carbachol) for serotonin and papaverine. pD2 values for carbachol, serotonin, and papaverine.

* P < 0.05 statistically different from the control group, n = number of animals.
KCl leads to contraction through membrane depolarization and influx of extracellular calcium through voltage-dependent calcium channels. Elevated intracellular calcium binding to calmodulin greatly stimulates myosin light chain kinase, so it initiates the contractile machinery [12, 13]. Therefore, decreased contractile response to KCl stimulation might be related to impaired voltage-dependent calcium channels in the CE group. Carbachol binds to muscarinic receptors, stimulating a G protein to activate phospholipase C, so activates protein kinase C and mobilizes intracellular calcium, leading to smooth muscle contraction [12]. Also, development of decreasing force in response to receptor activation of the injured tissues is a complex event that may be dependent on factors both at the level of the cell membrane and number of the receptors, receptor sensitivity, or postreceptor mechanisms. Abnormalities in one or more of these pathways might explain decreased responsiveness to carbachol after caustic esophageal injury.

**FIG. 2.** KCl-induced contractile responses. All points show the mean ± SEM of responses obtained from individual experiments on different tissues from different animals. Each point is expressed as mg of tension per mg of tissue wet weight. Number in parenthesis indicates the number of preparations used from different animals. *P < 0.05 compared with the control group.

**FIG. 3.** Concentration–response curves for serotonin in esophageal strips precontracted with carbachol (10⁻⁶–3.10⁻⁴ M). All points show the mean ± SEM of responses obtained in individual experiments on different tissues from different animals. Data are expressed as a percentage of the contraction induced by carbachol. Number in parenthesis indicates the number of preparations used from different animals. *P < 0.05 compared with the control group.
Serotonin binds to serotonergic receptors on esophageal smooth muscle cells, enhancing cAMP, and it leads to smooth muscle relaxation [14]. Serotonin-induced relaxant responses were significantly decreased whereas no significant changes were observed in papaverine-induced relaxant responses (nonreceptor-mediated mechanism) in the CE group. Therefore, it could be assumed that the decreased responsiveness to serotonin in the CE group might be attributable to impaired signal transduction. In conclusion, impaired smooth muscle reactivity above the fibrotic segment at least in part may play a role for esophageal motor dysfunction after caustic injury.

REFERENCES