Effect of repeated cycles of acute esophagitis and healing on esophageal peristalsis, tone, and length

X. Zhang, K. Geboes, I. Depoortere, J. Tack, J. Janssens, and D. Sifrim. Effect of repeated cycles of acute esophagitis and healing on esophageal peristalsis, tone, and length. Am J Physiol Gastrointest Liver Physiol 288: G1339–G1346, 2005. First published January 6, 2005; doi:10.1152/ajpgi.00492.2004.—Severe esophagitis is associated with motor abnormalities in the esophageal body and lower esophageal sphincter. Reflux disease involves repeated episodes of mucosal inflammation and spontaneous or treatment-induced healing. The aims of this study were 1) to further assess changes induced by acute esophagitis on esophageal peristalsis, tone, and shortening and 2) to assess the effect of repeated sequences of acute esophagitis-healing on these motor parameters. Experiments were performed on adult cats. Esophageal manometry and barostat were performed before, 24 h after, and every 7 days after intraesophageal acid perfusion (0.1 N HCl, 80 min). Esophageal length was measured during manometry, and compliance of the esophageal body was assessed with barostat. The identical protocol was performed 8 and 16 wk after the first acid perfusion. The degree of esophageal mucosal damage was evaluated by endoscopy, histopathology, and myeloperoxidase activity. Acid perfusion induced severe esophagitis. At 24 h, distal peristaltic contractions disappeared, lower esophageal sphincter pressure was reduced by 60%, the esophagus length was 1–2 cm shorter, and esophageal compliance was reduced by 30%. Most parameters recovered in 4 wk. Subsequent repeated acute injuries induced similar endoscopic esophagitis but a different pattern of inflammatory infiltration and fibrosis in the mucosa and muscle layers, resulting in milder motor disturbances. Acute experimental esophagitis provokes severe but reversible hypomotility. Spaced repeated acute injuries provoke milder motor effects, suggesting an adaptive response.

Patients with erosive gastroesophageal reflux disease (GERD) have repeated episodes of mucosal inflammation and spontaneous or treatment-induced healing. Hypotensive lower esophageal sphincter (LES) and abnormal peristaltic contractions in the esophageal body are more frequent in this group of patients than in those with nonerosive GERD (4, 13, 15, 25). The prevalence of peristaltic abnormalities increases with severity of mucosal damage, affecting 20% of patients with nonerosive GERD and up to 48% of patients with severe ulcerative esophagitis (13).

Esophagitis can also be associated with changes in esophageal tone and length. Patients with a chronic history of severe GERD and esophageal aperistalsis have a very compliant esophagus (19), whereas patients with less severe GERD may have normal esophageal tone (12). Intraesophageal acid perfusion provokes an inflammation-related contraction of the longitudinal muscle and esophageal shortening. This phenomenon has been suggested to participate in the development of hiatal hernias (5, 21).

The relation between esophagitis and motility has been studied using experimental animal models. In cats and opossums, acid perfusion-induced acute esophagitis provokes failure of primary peristalsis, frequent spontaneous contractions (16, 24), esophageal shortening (21), and reduced LES pressure (7, 23).

Although histological examination of acute experimental esophagitis showed changes and inflammatory cells similar to those found in biopsies from GERD patients (9), the evolution and reversibility of the associated motility changes might be different. In patients with GERD, endoscopically determined mucosal healing after medical or surgical treatment is not associated with complete recovery of esophageal dysmotility (27), suggesting a secondary, irreversible motor abnormality or a primary phenomenon.

In animal experimental acute esophagitis, however, LES pressure returned to normal (7) and acid-induced esophageal shortening was reversible on resolution of the inflammation (21, 29).

The magnitude and reversibility of esophagitis-induced motor disorder probably depend on the depth of injury within the esophageal wall, the involvement of peripheral neural control of motility and/or esophageal muscle layers, the inflammatory mechanism that is triggered, and the type of healing or restitution process. For example, patients with scleroderma have a severe defect in peristalsis due to replacement of esophageal muscle with fibrous connective tissue (20), whereas patients with moderate esophagitis may have ineffective esophageal motility that reverses after appropriate cholinergic stimulation with edrophonium (6).

Most experimental studies have assessed esophageal motor changes associated with an acute single esophagitis event. We hypothesized that repeated episodes of acute experimental esophagitis would lead to a progressive irreversible impairment of esophageal motor function similar to that observed in patients with severe GERD.

The aims of this study were 1) to further assess the effect of acute esophagitis on esophageal peristalsis, tone, and shortening, 2) to follow up the changes in esophageal motility induced by acute esophagitis until healing, and 3) to assess the effect of repeated sequences of acute esophagitis-healing on these motor parameters.
METHODS

The experimental protocol followed the “Guiding Principles for Research Involving Animals” described in the Declaration of Helsinki and was approved by the Ethical Committee for Experimental Animals of Catholic University Leuven.

Acute Esophagitis Model

Acute esophagitis was induced by intraluminal acid perfusion in adult cats of either gender (3–5 kg body wt). The animals were fasted overnight, and anesthesia was induced with ketamine chloride (Parke-Davis, Warner-Lambert, Zaventem, Belgium) at 15 mg/kg im for induction and 10 mg/kg im every 30–45 min for maintenance. Animals were positioned in the right lateral decubitus position with the head elevated to minimize bronchi aspiration. Intragastric perfusion of a 0.1 N HCl solution was performed at 1.0 ml/min during 80 min at 1 cm above the manometrically identified LES. Buprenorphine (Temgesic, Schering-Plough, Brussels, Belgium; 0.3 mg/day) was given as an analgesic.

The degree of esophageal mucosal damage was evaluated endoscopically (Olympus CF type 1301) in all the cats. Digital photographs were always taken for reassessment. Endoscopic classification of feline esophagitis was adapted from the Savary-Miller grading system.

Histopathological analysis was used to further evaluate the degree of acute inflammation and recovery. Evaluation of lesions was performed on samples obtained during autopsy. A 1-cm-long segment of the distal esophagus and the entire proximal stomach were excised in one piece after careful in situ inspection of the gastroesophageal junction. The whole sample was immersed in formalin and, after fixation, cut into two pieces along the longitudinal axis. Both pieces were entirely embedded in paraffin. Transverse sections (5 μm) were cut by step section and subjected to hematoxylin-and-eosin and von Gieson staining. The latter was used for assessment of fibrosis.

The inflammatory reaction was scored for distribution, composition, and intensity of different types of inflammatory cells. Distribution was evaluated according to the presence of inflammatory cells in the different layers of the esophageal wall and their presence with a complete circular (grade 2) or incomplete (<50% of the diameter, grade 1) distribution, which was considered a ratio of polymorphonuclear to mononuclear cells. The intensity was scored as follows: normal (0), mild increase (1), and severe increase (2).

Myeloperoxidase (MPO) is a plentiful constituent of neutrophils and serves as a marker of tissue neutrophil content. MPO activity in the mucosa and muscle was assessed spectrophotometrically (1). At 24 h after acid perfusion, the animals were killed and esophagi were removed. The esophageal mucosa and muscle were dissected sharply in cold 0.9% NaCl. Mucosa or muscle (100 mg) was homogenized in 3 ml of hexadecyltrimethylammonium bromide mixed with 50 mM potassium phosphate buffer (pH 6) and centrifuged at 12,500 g for 30 min at 4°C. The supernatant was then mixed with o-dianisidine dihydrochloride (0.167 mg/ml; Sigma Chemical) and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm was measured with a spectrophotometer (UVikon 810, Kontron Instruments). One unit of MPO was defined as that degrading 1 μmol of peroxide per minute at 25°C.

Esophageal Compliance

Esophageal compliance was calculated as the slope of the linear part of the volume-pressure relation. Manometric measurements were performed with a perfused multilumen catheter that included a 3-cm-long Dentsilve to monitor LES pressure and six side holes to record pressure changes in the gastric fundus and esophageal body (1, 2, 3, 4, and 8 cm above the LES). The assembly was perfused by a low-compliance pneumohydraulic perfusion system (Arndorfer Medical Specialties, Greendale, WI) at a flow rate of 0.4 ml/min and connected to external pressure transducers (Siemens Elema 746, Siemens, Iselin, NJ). The analog signal was digitized (model D1200AC, WinDaq Acquisition, DATAQ Instruments) and recorded onto a personal computer for further analysis.

Statistical Analysis

Values are means ± SE. Manometric and barostat parameters before and after each acid perfusion-induced occurrence of esophagitis were compared with paired t-test. The differences in motility parameters during repeated esophagitis events compared with baseline (before the 1st acid perfusion) were tested with one-way ANOVA followed by Dunnett’s multiple-comparison test. $P < 0.05$ was considered significant.
RESULTS

Esophagitis

Endoscopic findings. Before acid perfusion, the esophageal mucosa was normal in all animals (Fig. 2A). Of the 10 cats included in the repeated esophagitis-motility protocol, 8 developed grade III esophagitis (Fig. 2B) and 2 developed grade II esophagitis. Acute esophagitis 24 h after acid perfusion spanned 5–10 cm proximal to the mucosal gastroesophageal junction (Z line).

At 8 wk after the first acid perfusion, six cats showed complete endoscopic recovery (Fig. 2C) and four cats developed esophageal stenosis, provoking severe malnutrition, and were euthanized. Analysis of the esophagitis-motility relation is based on data from the six animals that completed the protocol.

The endoscopic findings after the second and third acid perfusions, performed at 8 and 16 wk, respectively, were identical to those observed after the first acid perfusion. Four cats developed grade III esophagitis, and two cats developed grade II esophagitis. Endoscopic control 4 wk after the last acid perfusion showed complete recovery in all six cats.

Histopathological findings. Samples obtained 24 h after the first acid perfusion showed necrosis of the mucosa covering 60% of the internal diameter (Fig. 3A). Only small islands of multilayered squamous epithelium could be detected. These islands were composed of two to three layers of cells, including the basal layer. The necrosis was transmucosal and involved large areas of the muscularis mucosa with complete loss of smooth muscle cells in some areas. Inflammatory cells were present in the mucosa, with a circular distribution involving 75% of the esophageal diameter reaching the level of the muscularis propria. In the mucosa, polymorphonuclear cells outnumbered mononuclear cells (2:1). The intensity of the cellular infiltrate (mainly located in the upper lamina propria under the epithelium) was graded as 2 (Fig. 3B).

The specimens obtained from four cats with endoscopic recovery 8 wk after the first occurrence of acute esophagitis showed a squamous epithelial lining composed of well-differentiated cells with normal stratification covering the entire surface. The lamina propria showed loss of papillary formations at the epithelial stromal junction in three cats. Cellularity was normal in all four animals, but fibrosis was noted in one. The most striking observations were noted in the muscularis mucosa and submucosa. In all the animals, the muscularis mucosa appeared irregularly thickened, with large areas of fibrosis between the smooth muscle cells and short areas of fibrotic interruptions characterized by a complete loss of smooth muscle cells. The submucosal connective tissue was variable, with more loosely arranged and more cellular areas and other areas of fibrosis or mature adipose tissue.

After the second acid perfusion (8 wk), the epithelial lesions were more extensive, with mucosal necrosis of 90% of the circumferential diameter (Fig. 3C), including vascular damage. The mucosal inflammatory reaction was limited to <50% of the circumference (grade 1). Mononuclear cells were as common as polymorphonuclear cells (1:1). The cells were mainly present in the deeper part of the lamina propria. No inflamma-

A

B

C

Fig. 1. Experimental protocol of acute esophagitis and repeated esophagitis-healing cycles. E, endoscopy; M, manometry; B, barostat.

Fig. 2. Endoscopic findings. A: normal mucosa before acid perfusion. B: severe esophagitis 24 h after 1st acid perfusion. C: normal mucosa 8 wk after 1st acid perfusion.
tory reaction was seen at the level of the muscularis propria (Fig. 3D). In summary, the inflammatory reaction after the second perfusion involved less tissue, the intensity was less pronounced, and the muscularis propria was spared.

MPO activity. Despite identical endoscopic esophagitis severity, MPO activity was significantly higher at the mucosal and muscle layers after the first acid perfusion (10.5 ± 1.9 vs. 1.9 ± 0.2 MPO units/mg) than after the second acid perfusion (3.1 ± 0.5 vs. 0.2 ± 0.1 MPO units/mg).

Esophageal Motility

Phasic peristaltic contractions. Before the first acid perfusion, peristalsis contractions traveling the whole esophageal body occurred at a rate of 5 ± 0.7 per 10 min. The mean amplitude of contractions at 2 cm proximal to the LES was 146 ± 20 mmHg. At 24 h after induction of the first occurrence of acute esophagitis, the number of peristaltic sequences was significantly reduced to 2.7 ± 0.2 per 10 min, with contractions in the distal esophagus dropping out in 60% of the sequences (Fig. 4A). When present, the mean amplitude of contractions at 2 cm above the LES was 10 ± 7 mmHg in two cats, but distal contractions were completely abolished in the other four cats.

The effect of the second and third acid perfusions on peristaltic contractions was significantly less marked than the effect observed after the first acid perfusion (Table 1, Fig. 4B).

LES pressure. Before the first acid perfusion, LES pressure was 62 ± 16 mmHg, with complete relaxation (95 ± 2%) after spontaneous swallowing. At 24 h after induction of the first occurrence of acute esophagitis, LES pressure was reduced by 60% (to 16 ± 5 mmHg) and LES relaxation was preserved (98 ± 2%, \( P > 0.05 \)). Complete recovery of basal LES pressure was observed in all animals in 4 wk. The effect of the second and third perfusions on LES pressure was less marked than the effect observed after the first acid perfusion (Table 1, Fig. 4C).

Longitudinal muscle contraction and esophageal shortening. The basal mean esophageal length (from LES to upper esophageal sphincter) was 17.2 ± 0.5 cm. After the first acid perfusion, the esophagus shortened in all cats by 7.8 ± 1.3%. Esophageal length was completely restored in 4 wk in all cats. The response to the second and third perfusions was less marked (Table 1) but not uniform. After the second perfusion, three cats showed no esophageal shortening and three cats showed shortening of 7.3 ± 1.6%. In these animals, esophageal length did not recover completely by 7 wk. After the third perfusion, two cats showed no esophageal shortening and four cats showed shortening of 6.0 ± 0.1%. In the final control, the esophagus recovered its normal length in four cats and remained shorter in two cats (5.9 ± 0.3%; Fig. 5B).
Esophageal tone. Before esophagitis, the mean basal esophageal tone (resistance to initial stretch) was $-0.81 \pm 0.43$ mmHg and esophageal compliance was $0.58 \pm 0.03$ ml/mmHg. Acute esophagitis induced an increase in tone and a less compliant esophagus (Fig. 6A). At 24 h after the first perfusion, the resistance of the esophageal wall to initial stretch increased to $1.17 \pm 0.75$ mmHg and the esophageal body compliance decreased to $0.41 \pm 0.07$ ml/mmHg ($P<0.05$). Despite mucosal endoscopic recovery and recuperation of other motility changes, esophageal compliance remained low at 7 wk (Fig. 6B). The second and third perfusions less markedly increased the esophageal resistance to stretch (Table 1). Similarly, esophageal compliance was less markedly decreased by the second and third perfusions (Table 1). In the final control, both parameters of esophageal tone were normal.

**DISCUSSION**

Esophageal motor dysfunction is frequent in patients with severe esophagitis and might contribute to a vicious circle that increases reflux, impairs acid clearance, and in turn aggravates esophagitis. The natural history of the esophagitis-associated motor disorder is still unclear. It is controversial whether it is an irreversible secondary effect of esophagitis or a primary phenomenon (8, 14, 27). To better characterize the relation between esophagitis and esophageal dysmotility, this study was designed to follow up spontaneous motility changes in an animal model of severe acute esophagitis until spontaneous mucosal healing and to assess the effect of repeated sequences of acute esophagitis-healing on these motor parameters. Acute experimental esophagitis decreased phasic contractions and LES pressure, provoked esophageal shortening, and increased esophageal tone. In the absence of a new injury, most of these motility changes were reversible. Subsequent repeated acute injuries induced similar endoscopic esophagitis but a different pattern of inflammatory infiltration and fibrosis, resulting in milder motor disturbances.

In agreement with previous studies, we found that acute esophagitis significantly reduced the frequency and amplitude of peristaltic contractions in the esophagus (7, 16, 24). Previous animal studies used acute experiments and did not follow up on the spontaneous evolution of motility changes. In our study, we describe for the first time a progressive recovery of esophageal contractions, which is complete by 4 wk. Previous in vitro studies suggested that acute esophagitis dysmotility is mainly due to abnormal neural modulation (11, 23, 26). Inflammatory mediators, such as interleukin-6 and platelet-activating factor, produced during acute esophagitis, can diffuse through the esophageal wall and reduce acetylcholine release from excitatory myenteric neurons to circular smooth muscle (2, 3).

Interestingly, a subgroup of patients with moderate esophagitis may have ineffective esophageal motility that reverses after appropriate cholinergic stimulation with edrophonium (6).

**Table 1. Esophageal motor function before and after repeated acid perfusion-induced esophagitis**

<table>
<thead>
<tr>
<th></th>
<th>1st Acid Perfusion</th>
<th>2nd Acid Perfusion</th>
<th>3rd Acid Perfusion</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>%Change</td>
</tr>
<tr>
<td><strong>Amplitude, mmHg</strong></td>
<td>146±20</td>
<td>10±7†</td>
<td>92±5</td>
</tr>
<tr>
<td><strong>Compliance, ml/mmHg</strong></td>
<td>0.58±0.03</td>
<td>0.41±0.07*†</td>
<td>31±11</td>
</tr>
<tr>
<td><strong>Length, cm</strong></td>
<td>17.2±0.5</td>
<td>15.8±0.6*†</td>
<td>8±1</td>
</tr>
<tr>
<td><strong>LES Pressure, mmHg</strong></td>
<td>62±16</td>
<td>16±5†</td>
<td>66±9</td>
</tr>
</tbody>
</table>

Values are means ± SE. LESP, lower esophageal sphincter pressure. *$P<0.05$ vs. Before (paired t-test). †$P<0.05$ vs. Before (1-way ANOVA). ‡$P<0.05$ vs. 1st acid perfusion (paired t-test).
As in previous studies, basal LES pressure was dramatically reduced with acute esophagitis and recovered to normal values by 4 wk. The swallow-associated LES relaxation, however, was preserved, confirming that acute esophagitis mainly affects cholinergic excitation of the LES smooth muscle but neural inhibition remains intact (23).

In our study, acute esophagitis was associated with esophageal shortening of ~8%, which is comparable with previous observations in opossums (21). The change in esophageal length is due to an active contraction of the longitudinal muscle layer, rather than structural fibrosis. With acute acid exposure, mast cell-derived mediators induce contraction of a hyperresponsive longitudinal muscle (22, 28). In the opossum, this contraction started to reverse very soon after the end of acid perfusion (21). In our model, the control at 4 wk after the first episode of acute esophagitis showed complete normalization of esophageal length.

Immediately after acute esophagitis, the esophageal body became stiffer; i.e., resting esophageal tone was increased, and esophageal compliance was decreased. Previous animal studies with acute experimental esophagitis demonstrated an increased intraesophageal baseline pressure (16) or a high baseline tension in muscle strips from the esophageal body (24). To the best of our knowledge, this is the first description of esophageal tone changes, measured with a barostat, during acute esophagitis. This technique has been used to assess esophageal tone in normal animals (17, 30) and healthy human volunteers (18). Cholinergic excitatory input seems to be the main control mechanism (30).

In our study, the increased esophageal tone was present even in the absence of endoscopically detectable inflammation with edema. This is in contrast with the reduced amplitude of circular peristaltic contractions but parallels the increased contractility of the longitudinal muscle layer, suggesting that the increased esophageal stiffness might be related to a tonic contraction of the longitudinal muscle.

In patients with severe reflux esophagitis, the motor abnormalities rarely recover after endoscopic mucosal healing. We hypothesized that repeated acute experimental esophagitis would lead to a progressive irreversible impairment of esophageal motor function similar to that observed in humans. Our model failed to simulate human chronic severe esophagitis. Not only were the motor disorders not perpetuated, but they were attenuated after the first acute esophagitis-healing sequence, suggesting that acute esophagitis might have induced an adaptive or protective mechanism. In patients with GERD, with severe esophagitis and irreversible dysmotility, such a mechanism might be absent or might be present but overcome by repeated injuries at short intervals. Interestingly, the reversible motor changes observed in our model were similar to those described after acute caustic esophagitis in humans (10).

Although motor abnormalities as a whole were less marked after repeated cycles of esophagitis, two of the six cats had persistent esophageal shortening at the end of the protocol. Recent studies in opossums suggested that such a mechanism might underlie the formation of hiatal hernias (21).
In our model, we observed a milder motor impairment after the second and third episodes of acute esophagitis. The difference in response to repeated identical protocols of acid perfusion might be located at the mucosal level (reduced inflammatory infiltration), between the mucosa and muscle layers (impaired diffusion of acid and inflammatory factors), or at the neuromuscular level (adaptive response to inflammatory mediators).

Our histopathological results suggest a reduced inflammation at the mucosal, submucosal, and muscle layers. Further studies are needed to exclude an adaptive response to inflammatory mediators at the neuromuscular level.

Although the mucosal endoscopic appearance was similar after the three acid perfusions (in length and degree of esophagitis), the histology and inflammatory reaction were different. The injury after the first and second acid perfusions was extensive, involving the entire depth of the mucosa and the major part of the surface. However, with the second acid perfusion, the inflammatory infiltrate was less intense, i.e., more mononuclear cells were present and mainly located in the deeper part of the lamina propria. Furthermore, the inflammatory reaction was less pronounced or was absent at the level of the muscularis propria after the second acid perfusion. The lower level of MPO activity after the second acid perfusion might be partially explained by the difference in the inflammatory reaction.

Despite endoscopic appearance of complete healing 8 wk after the first occurrence of acute esophagitis, histological examination showed fibrosis of the lamina propria and muscularis mucosa with loss of smooth muscle cells and, to a lesser extent, changes in the submucosa. These changes could partially explain the milder motility changes observed after the second and third episodes of esophagitis. The stromal papillae of the lamina propria were no longer present. These papillae usually contain small blood vessels. Leukocytes, involved in the inflammatory reaction after luminal aggression, are recruited from these vessels. The alterations of the connective tissue of the lamina propria could therefore be responsible for a reduced inflammatory reaction with a reduced production of cytokines; furthermore, the increased fibrosis could influence migration of inflammatory cells and cytokines toward the muscularis propria and the myenteric plexus.

In summary, acute experimental esophagitis decreases phasic contractions and LES pressure, provokes esophageal shortening, and increases esophageal tone. In the absence of a new injury, all these motility changes are reversible. Subsequent repeated acute injuries induce similar endoscopic esophagitis but a different pattern of inflammatory infiltration and fibrosis, resulting in milder motor disturbances, suggesting an adaptive or protective mechanism.

GRANTS

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REFERENCES


