MECHANISMS UNDERLYING THE DEVELOPMENT OF GASTROESOPHAGEAL REFLUX DISEASE

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SUMMARY

• *In recent years, a large deal of new information accumulated concerning the pathogenesis, diagnosis, and therapy of gatroesophageal reflux disease (GERD). Gastric contents, mucosal resistance and clearing, and gastric emptying, along with incompetence of the lower esophageal sphincter, are now recognized as contributing factors to the development of GERD. In this review, the discussion is concentrated on the diagnostic tests for detecting GERD and their accuracy as well as on the potential mechanisms underlying the development of GERD. (Biomed Rev 1997; 8: 101-109)*

• Gastroesophageal reflux disease (GERD) is a common foregut disorder with an estimated prevalence of 0,36% that accounts for approximately 75% of esophageal pathology (1). In the past, reflux symptoms were often attributed to a hiatus hernia (HH) (2). The presence and size of HH may affect the lower esophageal sphincter (LES) function followed by increased esophageal acid exposure and delayed acid clearance (3, 4). The latter is associated with more frequent episodes of nocturnal gastroesophageal reflux (GER) en-

hancing the development of esophageal lesions (5). However, it is now clearly established that pathological GER and HH usually exist as separate conditions. Whereas approximately 80% of patients with pathological GER as measured by an abnormal 24-hour pH test of the esophagus have a radiologically demonstrated HH, only 5-9% of HH patients show endoscopic evidence of reflux-esophagitis (RE) (6,7). Radiology and endoscopy, with or without biopsy, may reveal specific for acid reflux GER and RE, but are of poor sensitivity (8). The finding of endoscopic esophagitis suggests a high probability of GERD but does not automatically indicate its presence. Vomiting, nasogastric tubes, fungal or viral infections, and esophageal retention due to achalasia or a tumor can cause esophagitis (1,9). Consequently, a more current and appropriate definition of GERD is increased esophageal exposure to gastric juice (i.e. GER) with or without morphological damage of the esophagus (1). GERD predominantly includes abnormalities of esophageal and gastric function that give rise to symptoms prior to the development of mucosal lesions (9). The varied clinical manifestations of the disease underscore the importance of assessing and documenting the presence of pathological GER by means of specific pH and scintigraphic tests (10).

DIAGNOSTIC PROCEDURES FOR DETECTION OF PATHOLOGICAL GASTROESOPHAGEAL REFLUX

• *Definition and clinical manifestations of gastroesophageal reflux*

The typical GER patient complains of posture-related heart-

burn and regurgitation as a result of increased esophageal exposure to gastric juice with or without morphological damage of the esophagus (9). Odynophagia or dysphagia may occur in about 35-40% of GERD patients, mainly due to impaired esophageal motility in already developed RE (11). Esophageal spasm, stricture, or the presence of a large HH could also be a reason for demonstration of dysphagia (5,12). Only about 60% of classical GERD patients have evidence of mucosal damage, i.e. a presence of RE on endoscopy (9). Complications of GERD (erosive esophagitis, Barrett's esophagus or stricture) are particularly frequent and severe in patients who have a combined acid/alkaline GER (86%) as compared to those with acid reflux only (51%, p<0,01) (13, 14). The perfusion studies of Johnson and Harmon, and the *in vitro* experiments of Kivilaakso *et al* (reviewed in 13) had shown that both acid and bile can produce esophageal mucosal abnormalities such as action potential changes, hydrogen ion reflux, and permeability defects, which could produce consistent morphological lesions with clinical RE. The major injurious agent of acid refluxate is pepsin with optimal pH range for activity of 2 to 5, while the potentially injurious ingredients of duodenal juice are pancreatic enzymes like trypsin, lipase, and carboxypeptidase, which are activated in the pH range of 5 to 8 (15). Lanas *et al* (16) studied the adaptation of esophageal mucosa to acid- and pepsin-induced damage in rabbits and revealed that preexposure of the mucosa to acidified saline significantly decreased both the mucosal damage and the mucosal barrier dysfunction caused by acidified pepsin . This phenomenon was not related to cell proliferation but dependent, at least in part, on nitric oxide and epidermal growth factor (EGF) receptor-mediated pathways. A significant number of patients with excessive duodenogastric reflux had had previous foregut surgery, especially cholecystec-tomy or Billroth I gastrectomy resulting in a continuous flow of bile into the duodenum, which in turn may lead to GER thus promoting the development of GERD and RE (17, 18).

Functional tests and analysis of gastroesophageal reflux

GER can be directly detected and measured by gastroesophageal scintiscanning using nonabsorbable 99m Tc-sulfur colloid diluted in 300 mL of water as the patient is positioned under the collimator of a gamma-chamber (19). A reflux index is calculated as a percent of counts over the esophagus for 30-second intervals compared to counts initially present over the stomach, achieving a sensitivity and specificity of 90% (20). Because an indwelling catheter is not necessary, the study is particularly well tolerated by children and elderly patients (18, 19).

First described by Spencer in 1969, and popularized by DeMeester and Johnson, prolonged pH monitoring is now generally accepted as the most accurate method of assessing GER (9, 13, 21). An abnormal 24-hour pH test is a common finding in GERD patients with a sensitivity of 88%, and an excellent specificity of 98% (19, 22). The test is performed by positioning a pH electrode 5 cm above LES, and the probe is connected via a pH-meter to a strip chart recorder running at 15 crn/h (1). Reflux is diagnosed when the measured pH drops below 4. Patient reflux status is assessed by a composite score of 6 components: percent of acid exposure in the upright and recumbent positions, and reflux episodes-total number, number of those equal or longer than 5 minutes as well as the longest (23, 24). The development of miniature glass and ion (ISLET) electrodes and telemetry capsules, portable digital data recorders, and computer analysis software has made possible 24-hour pH monitoring to be performed as a outpatient study (25, 26).

DeMeester *et al* (1) reported variable patterns of GER. Some patients have excessive upright acid/alkaline exposure but normal recumbent exposure (upright refluxers), others have excessive acid exposure only when recumbent (supine refluxers), whereas a third group have excessive exposure in both postures (combined refluxers). Identification of these patterns, and correlation with endoscopy and histology has confirmed that night-time seems critical for the most detrimental reflux to occur (27, 28). The measurement of 24-hour pH has become a necessary study to confirm the presence of GERD in patients with typical as well as in those with atypical symptoms or other foregut disorder that could be confused with GERD (29-31). The presence of an abnormally high esophageal alkaline shift implies that the refluxed gastric juice contains bile or other constituents of the duodenal juice (16,32); the cumulative exposure of the esophagus to alkalinity was defined as abnormal when it exceeded 17,7%, i.e. 95th percentile of the data obtained in 50 normal volunteers (13, 33). Fuchs *et al* (34) have recently shown that duodenogastric reflux can also be quantified with 24-hour gastric pH monitoring, and appears related to increased esophageal exposure to pH over 7 recorded on esophageal pH monitoring. The authors also developed a scoring system for duodenogastric reflux using a large number of computer-generated statistical measurements, including the number and height of alkalinizing peaks, the baseline pH, the pH of the meal plateau, and the pattern of pH decline from the plateau. For the diagnosis of alkaline duodenogastric reflux and GER, more dangerous for the esophageal mucosa than acid reflux, simultaneous long-term measurements of gastric and esophageal pH are feasible and superior to esophageal pH monitoring alone (35-39). |

•   *Basics of morphological diagnosis ofreflux-esoph-agitis*

The problems with the morphological verification of GERD

are derived, at first, from the lack of parallelism between the endoscopic findings and the histological pattern (40). According to Johanssen *et al* and Csendes *et al* (9, 40), 60% and 54%, respectively, of the patients with clinically manifested GER reveal morphological changes, while esophagoscopic changes are minimal or entirely lacking. Second, the observed histological changes are not typical, and the diagnostic significance of the separate morphological features is controversial (41-43). Morphological changes are present both in the covering epithelium and in the lamina propria of esopha-geal mucosa. One of the earliest changes, although nonspecific, is basal cell hyperplasia of the mucosal epithelium (44). Basal cells comprise 15% or more of the thickness of the epithelial layer. The papillae of lamina propria are hyper-plastic and hypertrophic. They are elongated up to the middle third of the mucosal thickness, and may even reach the surface. Blood vessel congestion, and slight or more abundant mononuclear infiltration are present in the papillae. Hemorrhages resembling "lakes" can be found among the neutrophils and eosinophils around the papillae, and even diffusely among the epithelial cells. The parabasal layer cells show strong mitotic activity, while in the cells of stratum spinosum are observed balloon degeneration and significant reduction of glycogen. Interstitial edema as well as formation of superficial erosions or deeper ulcerations are present. Later, the bottom of ulcerations forms granulations, and the covering epithelium regenerates. Prolonged RE may lead in these regions to glandular metaplasia, and thus to Barrett's esophagus. Inflammatory infiltrates of different intensity consisting of lymphocytes, plasma cells, neutrophils, eosinophils, and histiocytes are present around the blood vessels or diffusely in the lamina propria. At a later stage, lymph follicles are formed, and fibrotic changes develop. Morphological evidence of mast cell degranulation was found in an animal model of acid-induced esophagitis (45), associated with increased intraluminal histamine and microvascular permeability. Ste-reologic analysis of electron micrographs revealed that within the mucosa, the mast cell average and nuclear areas as well as the area of intact granules were significantly reduced, which suggests that acid reflux exposure is associated with degranulation of esophageal mast cells, thus their mediators may play a role in the pathophysiology of RE. EOF receptors have been identified in the esophageal epithelium and on the surface of intracellular membranes of individual disaggregated esophageal cells (46), which suggests a possible role of EGF in the maintenance of epithelial integrity in the esophagus. The expression of EGF receptors was increased in inflamed esophageal mucosa associated with proliferating basal cells (47).

However, all these changes are not specific. Their expression varies, and different combinations of them are found in both patients with or without GER. Comparative studies (48, 49) demonstrated that epithelial changes dominate in the RE

patients, all of which showed epithelial cell balloon degeneration and glycogen reduction. The predominance of basal cell hyperpasia, papillary hypertrophy and hyperplasia, and intra-epithelial congestion and hemorrhages in the RE patients is statistically significant. The changes observed in lamina propria did not show a significant difference between the two groups. The inflammatory reaction was similar with slight preponderance of lymphocyte aggregates and follicles in the RE group. Unlike others (43), our opinion is that the presence of neutrophils and eosinophils in the epithelial layer and the lamina propria is not of diagnostic significance for RE, since these cells were equally present in both groups studied. Therefore, the morphological diagnosis of RE should be accepted after evaluation of all findings, especially the intra-epithelial lesions. Keeping in mind that these lesions are also present in esophagoscopically normal mucosa, it is evident that biopsy of lower esophageal segment is a compulsory dignostic test for all GER patients (48-51).

NATURAL BARRIERS TO REFLUX

- *Lower Esophageal Sphincter*

Although many factors may interact in maintaining gastroesophageal competence (Table 1), most investigators agree that LES appears the key component (9, 39, 52-54) . LES is defined as a 2-5 cm long region of elevated pressure with a range of 12 to 20 mm Hg that prevents retrograde flow of gastric contents into the lower esophagus (9, 52). Both the amplitude of pressure and the length of LES are important in maintaining competence (53, 55). A mechanically defective LES responsible for pathological GER is identified by means of manometry as having one or more of the following criteria: LES pressure of 6 mm Hg or less, overall LES length of less than 2 cm, or abdominal sphincter length of less than 1 cm (9). Resting LES pressure is probably maintained by a complex interaction of hormonal, myogenic, and neural mechanisms. Gastrin, motilin, gastrointestinal peptides, p-adrenergic an-

Table 1. *Potentially important components in prevention and pathogenesis of gastroesophageal reflux disease*

Defense mechanisms
Lower esophageal sphincter
Extrinsic mechanical factors
Esophageal mucosal resistance and clearance
Gastric emptying

Aggressive factors
Gastric acid
Pepsin
Duodenal contents (alkaline reflux)

tagonists, and oc-adrenergic agonists increase LES pressure, while cholecystokinin, estrogen, glucagon, progesterone, secretin, neuropeptides, and anticholinergics diminish LES pressure (56, 57). In 60% of the patients who have increased esophageal exposure to gastric juice, LES is mechanically defective, and these patients are unlikely to regain competence on drug therapy (9, 30, 57).

Most studies involving measurements of LES characteristics utilize a low-compliance manometric recording system using water-perfused poly vinyl 4-8 channel catheters by means of a stationary pull-through or rapid pull-through technique (1,25, 53, 55). The latter is more inaccurate because it does not take into consideration the variations of LES pressure due to respiratory or diaphragmatic crus contractions (58). These problems have been overcome by the use of 3-5 cm long water-perfused sleeve catheter that staddles the high pressure zone (59), thus allowing an accurate measurement of LES pressure for 24 hour or longer periods (60). Multichannel measurements exhibit a large recording variability, but their correlation with clinical conditions remains to be established. LES can be shown also by a digital three-dimensional computerized representation based on eight-channel pressure recordings data, which may facilitate qualitative interpretation of its profile and function (61). The introduction of modern computerized systems for the acquisition of manometric data gives the opportunity to assess the effects of the pressures radially exerted over the entire length of the sphincter, and integrates the length and pressure variables into a figure defined as LES pressure vector volume (62-64). The combination of 24-hour pH test and LES pressure monitoring has a specificity of 100% in identifying patients with pathological GER secondary to a defective sphincter (9, 30). In healthy controls with normal LES pressure reflux occurred only in the postprandial period during episodes of transient LES relaxation (58, 59, 65). Subsequent studies performed in patients with pathologic reflux showed that it occurred spontaneously, during a transient LES relaxation, or in response to intra-abdominal pressure rise or gastric distention (1, 52, 59, 66).

• *Extrinsic Mechanical Factors*

Prior to demonstrating the presence of LES, mechanical factors were thought to play the primary role in preventing reflux: the oblique angle of His, the diaphragmatic pinchcock action, the valve-like action of the intraabdominal esophagus, and the intraluminal mucosal rosette. All these together probably produce a "flap-valve" antireflux barrier (57). Both anatomical and physiological evidence demonstrates the importance of a lower esophageal segment held within the abdominal environment by the phrenoesophageal membrane, inserted normally about 3,3 cm above the junction of the tubular esophagus with the stomach (7). Although the importance of

extrinsic mechanical factors is secondary to LES function in the maintenance of esophageal competence, there is evidence showing that diaphragmatic crus fibers contribute to the high pressure zone at the LES (67-69).

ESOPHAGEAL CLEARANCE AND MUCOSAL RESISTANCE

•   The esophagus is cleared by peristaltic pressure waves, either after a swallow (primary peristalsis) or after a distention (secondary peristalsis). Although both forms of motor activity decrease the esophageal volume, primary peri- . stalsis is necessary for complete esophageal emptying (70). A combined scintigraphic and manometric study designed to examine the effect of contraction amplitude on the clearance of an acid bolus showed that both esophageal transit and clearance were delayed in GERD patients (71). These changes were associated with a decrease in the amplitude of esoph ageal contractions. Further, a close correlation between the degree of RE and peristaltic dysfunction has been demon strated (72). The acid refluxed into the distal esophagus ini tiated a series of segmented contractions rather than normal secondary peristalsis. This effect resulted in delayed acid clearing, and LES remained relaxed setting the stage for a further GER injury (73) (Fig. 1). Thus, GER itself may con tribute to episodes of poor and low LES pressure, and accel- , erate the development of RE and its complications - peptic stricture and Barrett's esophagus (2). Saliva (pH 6-7) also plays an important role in esophageal clearance acting as a buffer due to the high level of bicarbonates (47). The pre-. epithelial esophageal barrier is strengthened by the salivary organic components such as mucin, nonmucin proteins, sali vary prostaglandin E_2, and especially EOF. The rate of secre tion of mucin, nonmucin proteins, and EGF is impaired in GERD patients, whereas of prostaglandin E_2 remains essen tially unchanged (74, 75). Mastication in RE patients in creased salivary volume by 215%, EGF by 207%, prostaglan din E_2 by 240%, transforming growth factor-a by 225%, and viscosity by 64% compared to corresponding values in healthy controls (76).These data indicate that the impairment of EGF output from the esophageal salivary glands may have a detri mental impact on the protective potential of the esophageal mucosa, depressing the esophageal clearing and facilitating the development of RE. However, this opinion is disputed by Benamouzig *et al* who did not found increased EGF salivary concentration in GERD patients (77). The submandibular gland also secretes nerve growth factor (NGF), transforming growth factor-p, and kallikreins, which are secreted into the saliva and affect immune and mucosal tissues as well as nerve endings in the gastrointestinal tract. They are involved in the regulation of mucosal immunity, and in regeneration and healing of RE (78). The simultaneous release of EGF and NGF from the submandibular glands into the saliva and blood upon
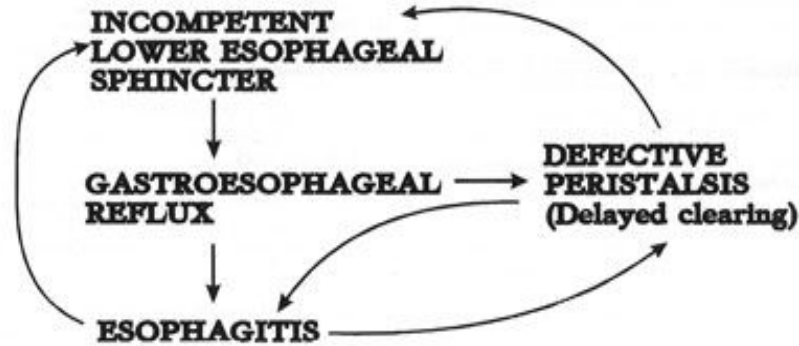
Figure 1. Schematic reprsentation of classical concept of the pathogenesis of gastroesophageal reflux disease, and various cyclic mechanisms of potential importance.

appropriate stimulation ensures coordination of the essential functions for controlling inflammation and initiating tissue repair in the gastrointestinal tract, and thus maintaining the integrity of esophageal mucosa (79, 80). A part of the protective effect of EGF is probably due to its ability to increase mucosal blood flow and potentiate sensory nerve transmission (81). Nevertheless, a dissociation between mucosal protection and microvascular perfusion has been noted (82).

GASTRIC VOLUME AND EMPTYING

• The role of gastric emptying in patients with symp tomatic GER remains controversial. GER due to gastric outlet obstruction is well known, and correctable by treating the gastric disorder (7). Early studies established that gastric distention results in a fourfold increase of the episodes of transient LES relaxations, but failed to demonstrate any dif ference in the rate of solid or liquid gastric emptying in GERD patients compared to controls (52, 59). Other studies have indicated a delayed gastric emptying of either liquids or solids in less than 50% of GERD patients (83). Analysis of antral contractility was performed in the same patients by manomet-ric recording assembly together with gastric scintiscanning using 500 mCi Tc-99m sulfur colloid meal. The migrating motor complex, a strong wave of contractile activity that sweeps through the stomach and small intestine, was associ ated with rise of LES pressure (84). Accordingly, diffuse motility disorders associated with impaired migrating motor complex generation were found to result in alterations of both

gastric emptying and LES pressure (57). Evaluation of gastric emptying on the basis of the postprandial alkalization of the gastric pH record is a new concept that evolved from multiple-probe gastric pH monitoring with simultaneous scintigraphic studies on gastric emptying (85). These studies showed that a typical meal caused a rapid pH rise in the gastric corpus compared to the baseline pH of 4 to 7. The high pH is maintained 10-20 minutes, the so called plateau period, and then rapidly falls to approximately 1 pH unit above the baseline followed by a period of slow decline to the interdigestive pH values. The postprandial pH profile of the gastric body closely correlated with the gastric emptying of a semisolid meal in these studies (85). The clinical use of 24-hour gastric pH monitoring was superior to O-diisopropyl imino-diacetic acid (DISIDA) scanning with cholecystokinin stimulation in detecting delayed gastric emptying and pathologic duodenogastric reflux (38). Delayed gastric emptying was confirmed scintigraphically in 85% of patients who had a prolonged postprandial alkalization of their gastric pH profile (38), suggesting that gastric 24-hour pH monitoring may be used to assess gastric emptying.

CONCLUSIONS

• The important factors involved in the development of GERD are LES incompetence, impaired esophageal clear ance and delayed gastric emptying. A question that remains to be answered is what factor is responsible for the progression of a physiologic phenomenon (gastric distention and post-

prandial reflux) to a pathologic condition (GERD). Early recognition of GERD requires redefining the disease on the basis of a measurable increase in esophageal acid/alkaline and gastric alkaline exposure during 24-hour pH monitoring.

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