Phospholipid composition of human gastric mucosa: a study of endoscopic biopsy specimens

G Nardone, P Lacetti, C Civiletti, G Budillon

Abstract
Gastric mucosal phospholipids, and in particular those of the surface layer, play an important part in mucosal barrier function. This study examined whether the phospholipid composition of the full thickness gastric mucosa is changed in peptic ulcer disease and gastritis. The phospholipid composition of gastric mucosa from endoscopic biopsy specimens in 28 subjects (8 healthy controls, 12 patients with duodenal ulcer, and 8 with chronic atrophic gastritis) was studied. In addition, the phospholipid composition of gastric mucosa was compared with that of duodenal mucosa in 10 patients with duodenal ulcer. As expected phosphatidylcholine and phosphatidylethanolamine prevailed in all three groups. Lysolecithin was the smallest component in the duodenal ulcer and chronic atrophic gastritis groups. The phosphatidylethanolamine value was higher in duodenal ulcer and lower in chronic atrophic gastritis compared with the control group. In chronic atrophic gastritis there was an appreciable amount of phosphatidylglycerol that was not present in patients with duodenal ulcer or in the control group. There was no significant difference in phospholipid composition between antral and duodenal sites in duodenal ulcer patients. In conclusion, the phospholipid composition of gastric mucosa changes in human gastrointestinal diseases but its relation to cellular functions needs further study.

Methods
Gastric endoscopies were performed with Fujinon UGI FP3, and biopsies with Fujinon K24 H forcesps. Precoated silica gel thin layer high resolution plates (Whatman HP-KF 10 x 10 cm) were used for chromatography. Phospholipid standards: lysolecithin (LL), sphingomyelin (SM), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE), cardiolipin (CL), phosphatidylinositol (PI), and phosphatidylglycerol (PG), were obtained from Sigma Chemical Co, Milan. The phospholipid chromatographic spots were detected by Phospay (molybdate ammonium, hydrochloric acid, mercury, sulfuric acid) supplied by Supelco Milano. The plates were developed with high performance thin layer chromatography and read with a scanner densitometer (Shimadzu CS 920). All chemicals were of reagent grade.

Subjects
The study population consisted of 28 patients (19 men and nine women; age range: 27–70) with dyspepsia who had not taken non-steroidal anti-inflammatory drugs and alcohol for at least one week before the study. Upper gastrointestinal endoscopy and biopsies were performed after mild sedation (N-butylbromuro joscina 20 mg and diazepam 10 mg). As a result of endoscopic and histological tests, eight subjects were classified normal (healthy controls with functional dyspepsia), 12 had duodenal ulcer disease, and the remaining eight had chronic atrophic gastritis. Microscopical examination with Giensa stain was negative for Helicobacter pylori contamination in all subjects. The study was approved by the local Ethics Committee and informed written consent was obtained from all subjects.

PHOSPHOLIPID ANALYSIS
The biopsy tissue specimens taken from the gastric antrum or the second half of the duodenum were collected to make a pool from each subject of about 25 mg (range 20–30 mg) of fresh tissue. The pools were homogenised in a Potter glass with chloroform/methanol (1:2 vol/vol), sonified for 10 minutes, and agitated overnight at 4°C as previously reported. Phospholipids were then extracted in chloroform/methanol (2:1 vol/vol) according to Folch. The extract was dried under a stream of nitrogen, redissolved in 30 µl...
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Figure 1: Standard phospholipid chromatography. Spots: 1: lysolecithin; 2: sphingomyelin; 3: phosphatidylcholine; 4: phosphatidylethanolamine; 5: phosphatidylinositol; 6: phosphatidylglycerol; 7: phosphatidylserine; 8: cardiolipin.

Figure 2: Gastric mucosa phospholipid chromatography of a normal subject. Spots: 1: lysolecithin; 2: sphingomyelin; 3: phosphatidylcholine; 4: phosphatidylethanolamine; 5: phosphatidylinositol; 6: phosphatidylethanolamine.

cloroform and spotted onto gel plates coated with silica (Whatman HP-KF 10 × 10 cm). Phospholipids were separated by two dimensional chromatography. The plates were developed for the first migration in a solvent saturated chamber consisting of chloroform/methanol/ammonium hydrate/water 184:105:7-5:7-5 vol and then dried in cold air. For the second migration, the plates were turned through 90° and the same solvents were used in a ratio of 120:160:5:5 vol. The phospholipid spots were detected by Phospray dye vaporisation and identified by comparing the migration of standard single phospholipids with their mixtures (Figs 1 and 2). Spots were read with the densitometer scanner at 360 nm and the phospholipid classes were quantified by comparing the densitometric values with standard curves of each phospholipid class (Fig 3). The reproducibility of this method was confirmed with two recovery tests, as follows. Gastric mucosa, obtained by pooling 10 antral endoscopic biopsy specimens (40 mg) from a single subject, was divided into two equivalent samples. Two equal mixtures containing the six phospholipid classes at known concentrations (about 10–15 µg of each class) were prepared simultaneously. The procedure described above (homogenisation, extraction, two dimensional chromatography, and densitometric reading) was carried out with the gastric mucosa sample (A), the phospholipid mixture (B), and the mucosa sample and phospholipid mixture together (C = A + B). Table 1 gives the results which are expressed as absolute mean values (µg) of two concordant experiments.
STATISTICAL ANALYSIS
Results are expressed as mean value (SEM). Data of independent groups (healthy controls, patients with duodenal ulcer, patients with chronic atrophic gastritis) were compared using analysis of variance to one channel and Scheffé multiple range tests. Differences were considered significant at p<0.01. Data of dependent groups (antral gastric and second portion duodenum in the same patient with duodenal ulcer) were compared using the paired Student’s t test. The overall data were examined using the SPSS/PC programme on an IBM PS2 personal computer.

Results
Tables II and III show the absolute and percentage values respectively of each phospholipid class from the gastric mucosa of the three groups studied (controls, patients with duodenal ulcer, and patients with gastritis). In each group there was a prevalence of phosphatidylcholine and phosphatidylethanolamine. In normal subjects the rank order of the various phospholipid classes was: PC>PE>PI>SM>LL>PS.

Patients with duodenal ulcer showed a significant increase, in both absolute and percentage values, in phosphatidylcholine, phosphatidylethanolamine, and phosphatidylglycerol, and a decrease in phosphatidylserine and lysolecinith compared with controls. The group of chronic atrophic gastritis patients had a reduction in total phospholipids and in lysolecithin, phosphatidylethanolamine and phosphatidylcholine, and an absolute and percentage increase in phosphatidylglycerol; variations in the other phospholipid classes were not significant. Phosphatidylglycerol was detectable in patients with chronic atrophic gastritis, but not in controls or in patients with duodenal ulcer. Figures 4 and 5 show the positive or negative percentage variation of absolute gastric phospholipid values in the duodenal ulcer and chronic atrophic gastritis patient groups v controls. There was no difference in the phospholipid composition between antral and duodenal sites in patients with duodenal ulcer (see Table IV).

Discussion
Recently, Schmitz and Renooij analysed the phospholipid composition of the gastric mucosa in surgical samples from four patients who had partial gastrectomy (Billroth II) for peptic ulcer. Despite differences in method and in the number of patients studied, their values for phospholipid composition (%) in patients with duodenal ulcer are very similar to ours. Using endoscopic biopsy we were able to compare patients with different diseases and control subjects with gastric mucosa histologically free of significant alterations. Our results confirm the constant prevalence of phosphatidylcholine and phosphatidylethanolamine, which together account for over 60% of all phospholipids identified in human gastric mucosa.

In a previous study we found a constant phospholipid composition in different parts of the stomach (fundus, corpus, and antrum). This study, showing no difference between antral and duodenal mucosa in patients with duodenal ulcer, confirms the earlier findings of a structural similarity with the phospholipid composition in the first part of the digestive tract. The overall evaluation of phospholipid composition compared with healthy controls showed that the lyssolecithin part becomes the lowest phospholipid component in both peptic ulcer and atrophic gastritis. The widest variations were seen in chronic atrophic gastritis: a lyssolecithin decrease and a phosphatidylethanolamine increase much greater than in duodenal ulcer. There was a small but significant increase in phosphatidylethanolamine in duodenal ulcer patients compared with controls, whereas the lipid component was consistently and significantly lower in chronic atrophic gastritis patients. Only the chronic atrophic gastritis patients showed some phosphatidylglycerol. In their study of patients with peptic ulcer, Schmitz and Renooij report the presence of cardiolipin in quantities comparable with the phosphatidylglycerol values we obtained in chronic atrophic gastritis. Results on phosphatidylglycerol and cardiolipin values should be viewed with some caution, however, because with the thin layer chromatography procedure these two fractions migrate in close juxtaposition (Fig 1).
It is difficult to interpret the variations in phospholipid composition found in our study. The cellular density of the inflammatory infiltrate in the mucosa may affect phospholipid composition, as has been reported for colon mucosa. The patients with chronic atrophic gastritis in our study had little or no inflammatory infiltrate, whereas all those with duodenal ulcer had a variable amount of inflammatory infiltrate in the gastric antrum. The difference in phosphatidylethanolamine values between the two patient groups could perhaps be related to the type of cellular rarefaction typical of chronic atrophic gastritis and to the difference in quantity of inflammatory infiltrate between the two groups.

The reduced LL/PC ratio and the increased PC/PE ratio in patients with chronic atrophic gastritis and duodenal ulcer is difficult to interpret in functional terms. It has been reported that, at least in the mucogel of the mucosal surface, the increase in phosphatidylcholine and phosphatidylethanolamine is related to the hydrophobicity and viscosity of the surface, although the chain length and the amount of saturation of the fatty acid side chain of phospholipids plays an even more important part in protecting mucosa against the back diffusion of hydrogen ions. The functional interpretation of our data, however, should be considered in the context that the contribution of surface phospholipid to the total phospholipid content of the biopsy specimens is relatively small.

In 1976, Orchard and Bickerstaff reported a reduction in all phospholipid classes, particularly phosphatidylcholine, in gastritis and especially in gastric ulcer patients compared with controls, whereas we found a significant phosphatidylcholine increase in both chronic atrophic gastritis and duodenal ulcer patients. The two studies cannot be compared, however, because Orchard and Bickerstaff did not define the type of gastritis because the patients in their study had ulcerative disease that affected the stomach and not the duodenum.

The results of this study provide evidence that phospholipid composition is changed in patients with gastroduodenal diseases. Little is known about the lipid metabolism in gastric mucosal cells and its relation to the cellular function; therefore, studies are required to determine the specific role of total mucosal phospholipid in gastric secretion and cytoprotective action. The importance of phospholipid adherence to the mucosal surface in gastric cytoprotection was first reported by Lichtenberger et al. The studies on gastric phospholipid composition and function, however, have been conducted on total thickness mucosa and in laboratory animals because it is difficult to obtain human mucosal biopsy specimens suitable for biochemical study. Hence, the analysis of phospholipid composition in endoscopic biopsy specimens such as those used in this study, provides a way to investigate the various phases of individual gastric diseases, and also allows investigations on the length and the saturation level of phospholipid linked fatty acid, and on the relation with the cell peculiarities of mucosa.

The authors thank Dr F D’Armiento for histological evaluation and Dr G Orlandi for statistical analysis. This paper was supported by a grant from MURST (1990–1991), Rome, Italy.

**Table IV** Phospholipids from duodenum and gastric antrum mucosa in patients with duodenal ulcer (μg/100 mg wet; mean values (SEM)).

<table>
<thead>
<tr>
<th>Phospholipids</th>
<th>Antrum (n=10) Mean (SEM)</th>
<th>Duodenum (n=10) Mean (SEM)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyssolecithin</td>
<td>15.4 (0-6)</td>
<td>14.4 (3-9)</td>
<td>NS</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>32.1 (2-7)</td>
<td>28.3 (3-5)</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>267.6 (10-4)</td>
<td>231.2 (11-0)</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphatidylserine</td>
<td>24.3 (2-4)</td>
<td>26.9 (3-2)</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>204.6 (9-4)</td>
<td>182.9 (7-7)</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphatidylinositol</td>
<td>78.8 (4-7)</td>
<td>78.0 (8-5)</td>
<td>NS</td>
</tr>
</tbody>
</table>

* p < 0.01 v controls (line 0)
18 Orchard JI, Bickerstaff CA. Gastric mucosal phospholipid concentrations in human patients with gastritis, gastric ulcer, and normals [Abstract]. Gastroenterology 1986; 90: 1573.
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Gut 1993 34: 456-460
doi: 10.1136/gut.34.4.456

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