Bile acid concentrations in the gastric juice of patients with erosive oesophagitis

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SUMMARY Intragastric total bile acid concentrations were measured before and after a corn oil test meal in 16 patients with erosive oesophagitis and symptoms of gastro-oesophageal reflux. Sixteen age and sex matched control subjects were also studied. No significant difference was detected between fasting or postprandial gastric bile acid concentrations in patients and in control subjects although a wide range of bile acid concentrations was detected among individuals in both groups. Gastric juice pH was <3.5 in seven patients when intragastric bile acid concentrations were >200 μmol/l. These results do not support a role for abnormal duodenogastric reflux in the pathogenesis of erosive oesophagitis. The detection of acid reflux in such patients during intra-oesophageal pH monitoring, however, does not exclude the presence of bile acids which may contribute to the cytotoxic potential of gastric juice.

Excessive reflux of gastric juice into the oesophagus has become accepted as the major determinant of reflux oesophagitis. Prolonged intra-oesophageal pH monitoring, however, has revealed imperfect correlation between the frequency and duration of acid reflux episodes and the severity of oesophageal mucosal damage.1,2

Several investigators have shown a marked cytotoxic effect of pancreatic and biliary secretions on oesophageal mucosa in laboratory animals.3-5 Conjugated bile acids, unlike pancreatic enzymes, continue to damage oesophageal epithelium in an acid medium.6-8 These observations have suggested a possible role for duodenogastric reflux of bile acids in the pathogenesis of reflux oesophagitis.

Although early intubation studies reported increased gastric bile acid concentrations in patients with hiatal hernia and oesophagitis,9-11 more recent non-invasive studies using radioactive biliary markers have suggested that duodenogastric reflux occurs rarely in patients with gastro-oesophageal reflux.12 13

We present a study of intragastric bile acid concentrations in patients with endoscopic evidence of erosive oesophagitis and in healthy control subjects, and discuss the relevance of our observations to the pathogenesis of reflux oesophagitis.

Methods

Patients

Sixteen patients (six women) whose ages ranged from 24–64 years (mean 48 years) were studied. All had symptoms of gastro-oesophageal reflux and had been found to have linear erosions in the lower third of the oesophagus during endoscopic examination. Eleven patients had a hiatal hernia detected at endoscopy. No patient was taking medication likely to cause oesophageal irritation and none gave a history of excessive alcohol consumption (>80 g ethanol daily). None was taking cimetidine or other treatment likely to alter gastroduodenal motility. No patient had previous gastrointestinal tract surgery (except appendicectomy), biliary tract surgery, or concomitant or previous peptic ulcer disease. Ultrasound scans or cholecystograms were carried out on all patients and those found to have gall stones were excluded from further study.

Sixteen healthy volunteers with no symptoms of gastrointestinal tract disease were also studied. Their ages ranged from 21–62 years (mean 45 years) and they were sex matched and age matched to within 10 years with the patients. All volunteers were shown to have no gall stones by preliminary ultrasound scanning of the abdomen.

Details of the reflux symptoms, smoking, and
alcohol ingestion were recorded for all patients and volunteers. Fifteen patients complained of heartburn, 13 of regurgitation, and seven of dysphagia. Only one was a cigarette smoker while six drank small to moderate quantities of alcohol. Five control subjects were cigarette smokers and 12 drank small to moderate quantities of alcohol. Informed written consent was obtained in every case and ethical committee approval was granted for the study.

Patients and volunteers had a size 14 Salem sump nasogastric tube passed into the stomach after an overnight fast. The position of the nasogastric tube was checked radiologically. After 45–60 minutes gastric juice contents were completely aspirated and discarded. Then, all gastric juice was collected over a 30 minute period using a suction pump and intermittent aspiration with a syringe. A test meal, containing 18 g corn oil and 12 g glucose, made up to 100 ml with warm water, was given through the nasogastric tube over a two minute period. Aliquots (10 ml) of gastric juice were aspirated at 20 and 40 minutes after the meal, and at 60 minutes the gastric contents were completely aspirated. All subjects were semirecumbent during the study, none complained of nausea or abdominal pain and none vomited.

Gastric juice samples were centrifuged at 1500 g and 4°C for 15 minutes to separate any corn oil or mucus from the aqueous portion. Bile acid concentrations and pH were measured in all samples.

The analysis of total bile acids was carried out using reagent mixtures, as described for the commercial kit produced by Nyegaard (Sterognost-3α Pho), which is based on the method of Fausa and Skalhegg.14 15 The technique depends on the conversion of bile acids by 3-α-hydroxysteroid dehydrogenase to corresponding ketosteroids with concomitant reduction of NAD to NADH. The NADH is produced mole for mole from bile acid and can readily be quantified using a spectrophotometer (Pye Unicam SP8-100) set to measure absorbance at 340 nM.

Determinations were made in triplicate using 100 µl samples of gastric juice at room temperature and at pH 9.5. Readings were taken after a reaction time of 20–40 minutes. Our assay was found to be linear up to a concentration of 1000 µmol/l of bile acid. Recovery of 5 µmol cholic acid from gastric juice (bile acid concentration, 108 µmol/l) was determined in 12 replicate samples to be 90–96%. The precision of the assay was assessed in gastric juice samples containing 108 µmol/l and 424 µmol/l bile acids. The coefficient of variation of 12 aliquots of the first sample was 3–7% and of 10 aliquots of the second sample was 2.7%. The smallest detectable difference of bile acid concentration was governed by the sensitivity of the spectrophotometer. For our instrument this was equivalent to 5 µmol/l of bile acid. The accuracy of the assay was unsatisfactory for bile acid concentrations less than 62.5 µmol/l.

Statistical analysis of the results was carried out using the Wilcoxon's signed rank test.

**Results**

Two patients and two asymptomatic volunteers had gastric juice of high pH (range 5.8–7.7) in all aspirated samples. All other subjects had one or more samples of pH 3.5 or less. Fasting and postprandial gastric juice pH values were compared between the patient group and the control group, no significant difference being detected (Table).

A wide interindividual range of gastric bile acid concentrations was observed in the patient group and in the control group. No significant difference was detected between fasting gastric bile acid concentrations in patients and in control subjects (Fig. 1). Furthermore, no significant difference was detected between gastric bile acid concentrations at 20, 40, and 60 minutes after the test meal in patients and in control subjects (Fig. 2). Peak postprandial bile acid concentrations were also compared between the two groups and no significant difference was observed.

Fasting or postprandial contamination of gastric juice with bile acids was observed in 12 of our patients although, in two of these, the bile acid concentrations were less than the lower limit of accurate detection of our assay (<62.5 µmol/l). In nine of the patients, bile acid concentrations were >200 µmol/l, an amount reported to be toxic to oesophageal mucosa when human oesophageal biopsies were incubated in a bile acid medium.6 Of these nine patients, seven had gastric juice of pH <3.5. Fourteen of our control volunteers had detectable intragastric bile acid, either fasting or postprandially, although in eight of these the concentrations were less than the lower limit of accurate detection of our assay (<62.5 µmol/l), and only five had concentrations of >200 µmol/l.

**Table. Gastric juice pH and volume (60 minutes postprandial) in patients and in control subjects**

<table>
<thead>
<tr>
<th>Analysis Type</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting pH</td>
<td>1.8 (1.0–7.3)</td>
<td>2.1 (1.1–7.7)</td>
</tr>
<tr>
<td>Mean postprandial pH</td>
<td>1.7 (1.2–6.9)</td>
<td>1.9 (0.8–7.2)</td>
</tr>
<tr>
<td>60 min postprandial vol (ml)</td>
<td>45 (27–175)</td>
<td>58 (10–130)</td>
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</tbody>
</table>
Bile acid concentrations in the gastric juice of patients with erosive oesophagitis

The volume of gastric fluid aspirated 60 minutes after the test meal was compared between the patient group and the control group, with no significant difference being detected (Table).

Discussion

Our results fail to confirm the occurrence of abnormal duodenogastric reflux in patients with erosive oesophagitis as described in earlier intubation studies. Unlike those previous investigations, our patients were a homogeneous group in that all had erosive oesophagitis, gall bladder disease was formally excluded and no patient had oesophageal stricture formation. Our data do not exclude the possibility that abnormal duodenogastric reflux of bile acid occurs and is of major importance in that group of patients who develop oesophageal strictures.

It has been argued that nasogastric tube placement induces uncoordinated gastroduodenal motility which permits duodenogastric reflux, but observation of radioactive biliary markers has suggested that this initial reflux is cleared from the stomach within one hour. Furthermore, a transpyloric tube has been shown recently to produce no disturbance in duodenogastric reflux in healthy human volunteers. To minimise the influence of intubation, we waited 45–60 minutes, then aspirated fully and discarded all gastric juice before collecting basal fasting samples. This precaution was not adopted in earlier studies and, hence, the claim from two reports that higher fasting bile acid concentrations were found in the gastric juice of patients with hiatal hernia, may be criticised.

Radioactive biliary markers have been used in two recent studies to assess duodenogastric reflux in patients with gastro-oesophageal reflux. Neither study revealed evidence of increased duodenogastric reflux. Matikainen and colleagues suggested that false positive results were produced by intubations, and that the non-invasive radioisotope scan was the appropriate means of investigating pyloric incompetence. We suggest that the choice of investigation is less clear cut. The value of a non-invasive method is undisputed, but the scintigraphic technique is only semiquantitative and does not detect small amounts of bile reflux unless very large doses of radioisotope are used. The levels of intragastric bile acids recorded in many of our patients and controls would have been associated with a negative radioisotope scan. Duodenogastric reflux is only one factor of several which contribute to the bile acid concentration in gastric juice since gastric emptying rate and secretory rate will also influence the bile acid concentration. If bile contributes to the development of oesophagitis, it is likely to be the bile acid concentration in gastric juice which determines its cytotoxicity rather than the presence of pyloric incompetence alone.

A delay in gastric emptying might prolong the contamination of gastric juice with bile acids. We did not observe any trend towards higher bile acid concentrations in the last postprandial samples from our patients with oesophagitis, and indeed, gastric juice volume measurements, at that stage, suggested that gastric emptying rates were similar in the two groups. Although delayed emptying of solid food has been recognised in patients with oesophagitis, conflicting reports on liquid emptying have appeared. Most investigators observing no abnormality. Nonetheless, it is possible that after ingestion of a more physiological solid-liquid meal, a delay in solid food emptying might influence the duration of postprandial gastric juice bile acid concentrations.

Fig. 1  Fasting intragastric bile acid concentrations in patients and in control subjects.
Patients Controls

Patients Controls

20 minutes 40 minutes 60 minutes

Fig. 2 Postprandial intragastric bile acid concentrations in patients and in control subjects.

collaboration.

Pellegrini and colleagues\textsuperscript{26} detected a group of patients with excessive alkaline reflux on 24 hour oesophageal pH monitoring and suggested that duodenogastric reflux was occurring in such patients. Almost all of our patients with detectable intragastric bile acids had gastric juice of pH <3.5, however, and entry of such fluid into the oesophagus would have been recorded as an episode of acid reflux. It is clear, therefore, that detection of acid reflux on oesophageal pH monitoring does not allow firm conclusions to be made about the cytotoxic component of gastric juice.

Experimental work with animals and with human oesophageal biopsies supports the concept that hydrochloric acid and bile acids, in combination, exert a more toxic effect on oesophageal epithelium than either agent alone.\textsuperscript{6,8} Unconjugated bile acids are harmless in an acid medium as they are precipitated out of solution. Most bile acids entering the duodenum are conjugated, however, and these remain in solution at acid pH, and can injure oesophageal mucosa.\textsuperscript{7} Conjugated bile acids at a concentration of 200 μmol/l and in an acid medium were shown to damage human oesophageal mucosal biopsies whereas hydrochloric acid alone produced little irritation.\textsuperscript{6}

We acknowledge that acid and pepsin are likely to be major cytotoxic constituents in gastric juice and our results provide no evidence for the occurrence of abnormal duodenogastric reflux in patients with oesophagitis when compared with controls. We have shown, however, that a sufficient intragastric concentration of bile acids is achieved in many of our patients, that bile may be a contributory factor to the development of oesophageal mucosal damage. Studies of the bile acid fractions in the gastric juice of patients with erosive oesophagitis, and in patients with oesophageal stricture, may give important information, as there is evidence that taurine conjugated bile acids are particularly toxic to oesophageal epithelium.\textsuperscript{7,8} Abnormal gastro-oesophageal reflux may occur not only post-prandially but also during the night when patients are recumbent.\textsuperscript{27} Analysis of bile acid concentrations in gastric juice samples taken through the
night would extend our knowledge of the cytotoxic potential of gastric juice and the patterns of duodenogastric reflux in patients with reflux oesophagitis.

Drugs which promote gastric emptying and bile acid chelating agents should reduce bile acid contamination of gastric juice and further assessment is required of such agents in the treatment of reflux oesophagitis.

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References