Oesophageal clearance of acid and bile: a combined radionuclide, pH, and Bilitec study

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Background: Studies combining pH and Bilitec monitoring found a high prevalence of both acid and duodeno-gastro-oesophageal reflux in severe reflux disease. Clearance of refluxed material is a major defence mechanism against reflux. Several studies have been devoted to oesophageal acid clearance but oesophageal clearance of refluxed duodenal contents (DC) has rarely been addressed.

Aim: To compare oesophageal acid and DC clearance.

Methods: Ten healthy volunteers (five women, mean age 23 (1) years) were studied. Firstly, a balloon tip catheter, positioned in the duodenum under fluoroscopy, was used to aspirate DC after stimulation by a high caloric liquid meal (200 ml, 300 kcal). During the second session, pH and Bilitec probes were positioned 5 cm above the lower oesophageal sphincter and a small infusion catheter was introduced into the proximal oesophagus. The subject was placed supine under a gamma camera. One of two different solutions (DC mixed with 0.2 mCi Tc^{99m} pertechnetate or citric acid (pH 2) mixed with 0.2 mCi Tc^{99m} pertechnetate) was infused into the proximal oesophagus and the subject was instructed to swallow at 20 second intervals. Clearance was assessed using scintigraphy (dynamic acquisition, one frame per second in the anterior view; calculation of time to clear peak counts to background level), pH (time to pH<4) or Bilitec (time absorbance >0.14) monitoring, with or without continuous saliva aspiration. Each condition was studied twice in a randomised design; measurement time was four minutes, interrupted by water flushing, with a two minute rest period. Results are given as mean (SEM) and were compared by Student's *t* test and Pearson correlation.

Results: Scintigraphic evaluation showed a volume clearance time of 29 (3) seconds for acid and 28 (9) seconds for DC (NS). Saliva aspiration had no significant influence on volume clearance of acid or DC (28 (4) and 30 (13) seconds, respectively; NS). pH monitoring showed an acid clearance time of 217 (15) seconds, which was significantly prolonged to 324 (30) seconds during saliva aspiration (*p*<0.05). Bilitec monitoring showed a DC clearance time of 131 (27) seconds, which was not significantly prolonged by saliva aspiration (176 (36) seconds; *p*=0.08). DC clearance was faster than acid clearance, either without or with saliva aspiration (*p*<0.055 and *p*<0.05, respectively).

Conclusions: Under experimental conditions, liquid acid and DC solutions have comparable volume clearances. Chemical clearance occurs slightly faster for DC than for acid, and saliva plays a major role in the clearance of acid only.

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Clearance of refluxed material is a major defence mechanism against mucosal damage caused by gastro-oesophageal reflux. Reflux of gastric contents into the oesophagus causes distension which triggers peristaltic bolus clearance of refluxed material into the stomach.¹ The importance of gastric acid in the pathophysiology of reflux disease is clearly established. Apart from peristaltic clearance, often referred to as volume clearance, so-called chemical clearance is an additional defence mechanism against acid reflux and consists of swallowed saliva and secretions of oesophageal glands. The presence of bicarbonate in these secretions is important in neutralising the aggressive effect of acid on the mucosa.²

Animal studies suggest that refluxed duodenal contents (DC), which contains bile acids, bile salts, and pancreatic juice, may act in synergy with acid to induce oesophageal lesions.^{3–5} Since the introduction of the fiberoptic spectrophotometer Bilitec probe, duodeno-gastro-oesophageal reflux (DGOR) can also be quantified in humans. Combined pH and Bilitec monitoring studies found a high prevalence of both acid reflux and DGOR, especially in severe reflux disease and Barrett's oesophagus.^{6–9} The mechanism by which DC is cleared from the oesophagus has not been studied extensively. The peristaltic volume clearance mechanism is likely to

have an effect on a bolus of DC refluxate but it is unclear whether other clearance mechanisms are also involved.

Moreover, bile reflux episodes measured with the Bilitec last significantly longer than acid reflux episodes measured with pH electrodes.^{8–10–11} One reason might be the absence of a chemical clearance mechanism for DC, in comparison with acid reflux. Another possible explanation might be the Bilitec probe configuration, which consists of a small cup, which may be prone to trapping of liquid.^{6–11}

Therefore, the aim of this study was to compare oesophageal acid and DC clearance and to evaluate the mechanisms involved in oesophageal clearance of DC.

MATERIALS AND METHODS

Healthy volunteers

Ten healthy volunteers (five women) with a mean age of 23 (1) years took part in this study. None of the volunteers had

Abbreviations: AVCT, acid volume clearance time; $\dot{C}_{max}$, maximum count activity; DC, duodenal contents; DCCT, duodenal contents clearance time; DCVCT, duodenal contents volume clearance time; DGOR, duodeno-gastro-oesophageal reflux; VCT, volume clearance time

symptoms or a history of reflux disease or of surgery to the upper gastrointestinal tract or thorax. No medication was used except oral contraceptives. All volunteers were known to have normal oesophageal motility based on a previous oesophageal manometry study. Volunteers were asked not to smoke on the morning of the test and not to eat at least 10 hours prior to the test. All volunteers gave written informed consent for the study, which was approved by the ethics committee of the hospital.

Duodenal contents aspiration

In the first session, DC were collected through a tube with a balloon, which was positioned in the second part of the duodenum under fluoroscopic control. The balloon serves to prevent mixing of gastric contents or food with DC. After the balloon was insufflated with air (fig 1), a high caloric drink was given (200 ml Nutridrink, Nutricia, Belgium; 300 kcal: 13% proteins, 48% carbohydrates, and 39% lipids). Aspiration was performed until approximately 20 ml were collected. Preliminary studies revealed that this was the maximal duodenal fluid volume that consistently could be aspirated under the conditions of the study. The fluid was divided into 4 ml portions and frozen at -80°C .

Analysis of duodenal fluid

The viscosity of the aspirated DC was determined at 37°C by capillary viscometer.¹² For characterisation of DC lipid composition, bile salt concentrations were determined employing the 3α -hydroxysteroid dehydrogenase assay.¹³ Total phospholipid levels were determined using phospholipase D and choline oxidase.^{14, 15}

pH, composition, and viscosity of the aspirated DC were analysed. Concentrations of bile salts, total lipids, phospholipids, and the bile salt/phospholipid ratio, as well as amylase and lipase concentrations were measured.

Figure 1 Scheme of the duodenal contents aspiration catheter with balloon insufflated after drinking a liquid meal of 200 ml (300 kcal).

Acid and duodenal fluid labelling

One hour before the scintigraphic investigation took place, the four portions of duodenal fluid were defrosted and every 4 ml mixed with 0.1 ml 0.20 mCi of $\text{Tc}^{99\text{m}}$ pertechnetate. The fluid was shielded from light by aluminium paper wrapped around the syringes.

Four portions of acidic fluid were made and consisted of citric acid, titrated to pH 2, mixed with 0.1 ml 0.20 mCi $\text{Tc}^{99\text{m}}$ pertechnetate per 4 ml. The total maximum exposure of the volunteer to labelled DC and acid was approximately 1 mSv.

pH monitoring

Oesophageal pH monitoring was performed using an antimony pH electrode with a separate skin reference electrode (Synectics Medical, Stockholm, Sweden). Data were stored on a portable digital recorder (Digitrapper Mk III; Synectics Medical). Before each study, the pH probe was calibrated in buffer solutions of pH 7 and 1.

Bilitec monitoring

The fibreoptic spectrophotometer Bilitec 2000 (Synectics) was used to quantify the presence of DC in the oesophagus. The system consists of a miniaturised probe of 1.5 mm in diameter that carries light signals into the oesophagus and back via a plastic fibreoptic bundle. Before each study, the probe was calibrated in water.

Study protocol

During the second session, pH and Bilitec probes were positioned 5 cm above the lower oesophageal sphincter, assessed by manometric investigation, and controlled by fluoroscopy. Fluoroscopic control never exceed three seconds, at a radiation exposure of $20 \mu\text{Sv/s}$. A small infusion catheter was introduced in the proximal oesophagus 4 cm past the upper oesophageal sphincter (fig 2). After positioning of the probes, a 60 minute accommodation period was provided prior to the start of the acquisitions. Subsequently, the

Figure 2 Scheme of the positioning of the pH and Bilitec probes, 5 cm above the lower oesophageal sphincter, and localisation of the infusion catheter approximately 4 cm below the upper oesophageal sphincter.

subject was placed in the supine position under a gamma camera to exclude the forces of gravity.

In each subject, a total of eight clearance measurements (four DC and four acid solution) were performed under combined fluoroscopic and pH/Bilitec monitoring. A 4 ml bolus of labelled fluid was introduced through the infusion catheter in 2–3 seconds and 1 ml of air was used to clear the catheter of fluid. Four boluses of DC were instilled, and each time subjects were asked to swallow every 20 seconds. During the second and fourth sessions, saliva was aspirated using a dentist's tube. The order of experiments was not randomised to maintain simplicity of the acquisition protocol. After every bolus clearance measurement, the volunteer drank 5 ml water to wash out the tracer fluid and to neutralise the oesophagus, and an additional two minute period of rest followed. Subsequently, the study protocol was repeated using four boluses of labelled acidic fluid. Shortly before each bolus administration a four minute planar dynamic acquisition period (240 frames; one second per frame) was started in the anterior view.

Data analysis

Bilitec and pH data were analysed with the aid of commercially available software (Gastrosoft Inc.; Syntectics Medical, Irvine, Texas, USA). Acid clearance time (ACT) was defined as the time needed to reach a pH >4 after the pH decrease below 4 induced by instillation of the acidic fluid bolus. Duodenal content clearance time (DCCT) was defined as the time to reach an absorbance of less than 0.14 after an initial increase in absorbance above 0.14 induced by instillation of the DC bolus.

Volume clearance times (VCT) were analysed by constructing dynamic time activity curves from the scintigraphic data. The oesophagus was divided along its longitudinal axis into three equal anatomical regions (proximal, medial, and distal) and scintigraphic measurements were analysed for the total oesophagus and for each section separately. For each bolus administration, the maximum count activity ($\dot{C}_{max}$) was determined. To rule out overlap with material accumulating in the proximal stomach, the range of interest was set manually (fig 3). In the beginning of the experiment, counts returned to the original baseline after bolus passage. With prolonged duration of the experiment, scatter from material pooled into the fundic area influenced return to baseline levels. To minimise the influence of this scattering, mean counts over the last 30 seconds of the measurement were calculated ($\dot{C}_{210-240}$), assuming complete oesophageal clearance at that time. VCT in seconds was defined as the time needed to reach a count intensity of $\dot{C}_{210-240}$ plus 25% of the maximum counts ($0.25 \dot{C}_{max}$) over the region of interest. Expressed as a formula: $VCT = \text{time } (\dot{C}_{210-240} + 0.25 \times \dot{C}_{max})$. Acid volume clearance time (AVCT) was defined as the time needed to clear a labelled acidic fluid bolus. DC volume clearance time (DCVCT) was defined as the time needed to clear a labelled DC bolus, using similar calculations.

Statistical analysis

Values are expressed as mean (SEM). Clearance times were compared using the Student's *t* test. The Pearson's correlation coefficient between different clearance times was calculated by linear regression analysis. A *p* value <0.05 was considered significant.

RESULTS

Fluid constitution

The mean pH of DC fluid was 6.7 (0.3). The fluid was representative of normal DC,¹² and showed a mean total lipid concentration of 0.58 (0.26) g/dl. Mean bile salt concentration was 9.3 (4.8) mM, and the mean phospholipid

Figure 3 Example of a scintigraphic analysis for the volume clearance of acidic and duodenal contents (DC) fluid, divided into four ranges of interest for the calculations: total, proximal, medial, and distal area. The x axis on each graph represents time in seconds.

concentration was 715 (228)mM, resulting in a molar phospholipid to bile salt ratio of 0.19 (0.16). The viscosity of the DC fluid was significantly higher than the viscosity of the acid solution (0.996 (0.05) v 0.7 (0) mPA·s; *p*<0.05). Levels of amylase and lipase activities in aspirated duodenal fluid were 72 800 (8000) U/l and 173 800 (89 400) U/l, respectively.

Scintigraphy

The volume clearance times for acid (AVCT) and DC (DCVCT) are summarised in table 1. AVCT and DCVCT did not differ significantly for total, proximal, medial, or distal areas of interest (fig 4). In the distal oesophagus, AVCT and DCVCT were significantly longer compared with the proximal oesophagus. The distal oesophageal AVCT and DCVCT were significantly correlated (*r*=0.75, *p*=0.01). All other segmental AVCT and DVCT were not significantly correlated at any level (all *r*<0.47; all *p*>0.05).

There was no significant correlation between fluid viscosity and volume clearance times without aspiration of saliva (*r*<0.01 and *r*=−0.08, respectively, for AVCT and DCVCT; both *p*>0.05).

pH and Bilitec monitoring

Mean ACT, determined by pH monitoring, was 217 (15) seconds, which was significantly longer than the mean DCCT of 131 (27) seconds, determined by Bilitec monitoring (*p*<0.05) (fig 5).

ACT and DCCT were not significantly correlated (*r*=0.55; *p*>0.05). Chemical clearance times, both for acid and DC, were significantly longer than volume clearance times (all *p*<0.005). A significant correlation was present between ACT and AVCT (*r*=0.74; *p*<0.05) and between DCCT and DCVCT

Table 1 Volume clearance times (mean (SEM)) for acidic fluid (AVCT) and duodenal contents (DCVCT), with and without saliva aspiration

	Not aspirated			Aspirated		
	AVCT (seconds)	DCVCT (seconds)	p Value	AVCT (seconds)	DCVCT (seconds)	p Value
Total	29.3 (0.8)	29.6 (3.6)	NS	27.8 (1.2)	30.4 (4.4)	NS
Proximal	22.4 (1.3)	19.8 (1.9)	NS	20.2 (1.9)	22.9 (2.3)	NS
Medial	25.8 (1.2)	24.1 (1.9)	NS	24.6 (1.1)	24.5 (1.7)	NS
Distal	28.5 (1.0)*	28.2 (2.8)*	NS	28.0 (1.4)*	29.9 (4.2)	NS

Volume clearance did not differ with and without aspiration for any of the parameters at any level.

*p<0.05 compared with proximal oesophagus.

($r = 0.78$; $p < 0.05$). There was no significant correlation between fluid viscosity and chemical clearance times without aspiration of saliva ($r < 0.01$ and $r = 0.017$, respectively, for ACT and DCCT; both $p > 0.05$).

Influence of saliva aspiration

Aspiration of saliva had no significant influence on volume clearance times (table 1, fig 3). DCVCT, but not AVCT, with and without saliva aspiration were significantly correlated (respectively $r = 0.79$, $p = 0.01$ and $r = -0.12$, $p > 0.05$).

Saliva aspiration caused a significant increase in ACT (from 217 (15) to 324 (30) seconds; $p < 0.05$) but did not significantly alter DCCT (131 (27) to 175 (36) seconds; $p > 0.05$) (fig 5). ACT with or without aspiration were not significantly correlated ($r = 0.31$, $p > 0.05$) but a significant correlation was found between DCCT with and without aspiration ($r = 0.75$, $p = 0.01$) and between DCCT and ACT with aspiration ($r = 0.74$, $p = 0.01$).

There was no significant correlation between fluid viscosity and volume clearance times with aspiration of saliva ($r < 0.01$ and $r = -0.36$, respectively, for AVCT and DCVCT; both $p > 0.05$).

DISCUSSION

Although several studies have been devoted to oesophageal acid clearance mechanisms,^{1 16 17} clearance of refluxed duodenal contents (DC) has not been addressed. By injecting radiolabelled acid fluid into the distal oesophagus, Helm *et al* demonstrated that elimination of acid from the oesophagus is a two step phenomenon. The bulk of the acid bolus was cleared by a secondary peristaltic wave, which was referred to as volume or bolus clearance. Clearance of the residual acidic fluid that sticks to the wall was significantly delayed by oral saliva aspiration, suggesting neutralisation of acid by swallowed saliva. This second step was referred to as chemical clearance.¹⁶ In keeping with these observations, Shaker *et al* showed that an increase in swallow frequency

accelerated the clearance of acid from the oesophageal mucosa.¹⁷ In addition, oesophageal mucosal defence mechanisms may also provide protection against noxious elements in the refluxate.^{18 19}

In the present study, we confirmed the findings of Helm *et al* with regard to the influence of saliva aspiration on chemical clearance and not on volume clearance of an acidic fluid bolus.¹⁶ The 4 ml boluses we used were smaller than the 15 ml boluses used by Helm *et al*, but they demonstrated that no significant difference was observed in clearance velocity using boluses of 2, 5, or 15 ml.¹⁶ It seems therefore that oesophageal peristalsis provides clearance of refluxed DC while peristalsis and chemical clearance provide clearance of refluxed acid.

Studies combining pH and Biletec monitoring in patients with severe oesophagitis and Barrett's oesophagus revealed that DGOR episodes last significantly longer than acid reflux episodes.^{8 10} The reasons underlying this difference have not been elucidated but could be related to a slower volume clearance of DGOR, the absence of a chemical clearance mechanism for DGOR, or to the physical characteristics of the Biletec probe, which may be prone to liquid entrapment in the tip. In addition, the biophysical characteristics and volume of the refluxate may be different for DGOR episodes compared with acid reflux episodes.

Using scintigraphy and instillation of labelled boluses, we did not find any difference in volume clearance between acid and DC fluid, for any segment of the oesophagus. Chemical clearance of a standardised DC fluid bolus measured with the Biletec probe was significantly shorter than chemical clearance of an acid bolus measured with a pH probe. As expected, saliva aspiration prolonged acid chemical clearance but did not affect DC clearance.

Figure 4 Comparison of acid and duodenal contents (DC) volume clearance (mean (SEM)), with and without saliva aspiration.

Figure 5 Comparison of acid and duodenal contents (DC) chemical clearance time (mean (SEM)), with and without saliva aspiration. *Significant difference.

The bile salt concentrations in the aspirated duodenal fluids are higher compared with concentrations commonly reported for oesophageal fluid in healthy volunteers as well as in patients with reflux disease.²⁰ This probably reflects the absence of a diluting effect of gastric juice to which the duodeno-gastro-oesophageal refluxate is normally exposed in patients without gastric surgery. When gastro-oesophageal reflux occurs, the refluxate will usually be a mixture of gastric acid, fluid from duodenal origin, and food. Our study provides information on the clearance time of two of these components in their purest form. As chemical clearance of acid was slower than chemical clearance of DC, our findings suggest that further dilution of DC by gastric juice might prolong chemical clearance times for DC, thereby promoting toxic effects of much lower concentrations of bile salts in the oesophagus.

The finding that DCCT was significantly shorter than ACT certainly argues against entrapment of liquid in the small cup at the tip of the Bilitec probe as a confounding factor. Because of the characteristics of the probes, it is in theory conceivable that the pH probe compared with the Bilitec probe more easily reaches small amounts of fluid adherent to the mucosa, although scintigraphy did not reveal any differences between both. If DC fluid is in the same way adherent to the mucosa, the Bilitec probe might even underestimate the presence of DC fluid in the oesophagus, but until now no in vivo experiments have provided evidence of this possibility.

The constitution of the fluid, including its viscosity, may also be a relevant factor explaining the difference in chemical clearance. In the present study, the internal cohesive forces of DC fluid were significantly stronger than those of acidic fluid, potentially allowing for more complete evacuation during peristalsis. However, no correlation was found between bolus or chemical clearance and viscosity of the acid or DC fluid, suggesting that viscosity was not the major determinant of clearance velocity. Handling of refluxate by the oesophageal mucosa is another potentially contributing factor. Bicarbonate secretion by oesophageal submucosal glands is likely to facilitate intraluminal neutralisation of acid.²¹ In addition, a small mucus layer, probably the result of mucus secretion in saliva and in oesophageal submucosal glands, also provides protection of the oesophageal mucosal cells.²² This surfactant layer may act to prevent direct contact between the cell membranes and lipophilic liquids, and differences in diffusion of fluid into this layer could be another explanation for the difference in clearance times between acidic and DC fluid.^{18, 19} Similar to the stomach,²³ the mucus layer on the oesophageal mucosa may not attract DC because of the lipophilic character whereas acid could more easily diffuse because of the hydrogen ion content.²⁴ If not neutralised, acid that has diffused into the mucus layer may be less readily cleared by peristalsis compared with acid present within the lumen of the oesophagus. Furthermore, if the pH probe is also embedded in the mucus, trapping of acid ions in this microenvironment may result in prolonged clearance times. The mucus layer may help to provide a barrier against adherence of bile salts to the cell membrane, which is thought to constitute the damaging effect of bile salts on the oesophageal mucosa.²⁵

The present clearance studies, which failed to demonstrate prolonged volume or chemical clearance for DC compared with acid, do not readily explain the longer duration of DGOR episodes during ambulatory monitoring. Previous analyses have shown that most DGOR episodes occur at acidic pH, and are in fact mixtures of acid and DGOR.⁸ It seems conceivable therefore that episodes of DGOR constitute a higher volume of refluxate than episodes of acid reflux alone, and that this

accounts for the longer clearance time of these episodes. Preliminary data, showing that patients with pathological exposure of the distal oesophagus to both acid and DGOR have more reflux in the proximal oesophagus than patients with pathological acid exposure alone, are in keeping with this hypothesis.²⁶ Further studies are needed to address this issue.

In conclusion, under experimental conditions, volume clearance of acidic fluid and of DC fluid is comparable and depends on oesophageal peristalsis. Chemical clearance of acid is slower than chemical clearance of DC. Neutralisation by saliva plays a major role in the chemical clearance of acid only. We found no evidence for entrapment of the DC bolus in the Bilitec probe, thereby artificially prolonging DGOR episodes. The physical characteristics of the liquid, especially viscosity, may influence peristaltic clearance.

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