Reappraisal of bicarbonate secretion by the human oesophagus

A Mertz-Nielsen, J Hillingsø, K Bukhave, J Rask-Madsen

Abstract

***Background and aims*—Administration of omeprazole to healthy volunteers was recently reported to increase proximal duodenal mucosal bicarbonate secretion. As human oesophagus also secretes bicarbonate, the hypothesis was tested that omeprazole may stimulate oesophageal bicarbonate secretion and thus contribute to the therapeutic efficacy of the drug in gastro-oesophageal reflux disease.**

***Subjects and methods*—In nine healthy volunteers, oesophageal "steady state" perfusion of a 10 cm open segment of distal oesophagus was performed twice in random order. The volunteers were pretreated with either 60 mg/day omeprazole for three days and 80 mg intravenous omeprazole before perfusion or 600 mg/day ranitidine for three days and 50 mg/h intravenously during the perfusion. Saliva and samples of aspirate from the perfused oesophagus and stomach were collected and bicarbonate concentrations were measured.**

***Results*—The median rates (95% confidence intervals) of intrinsic oesophageal bicarbonate secretion, corrected for contaminating salivary and gastric bicarbonate, were 89 (33–150) and 121 (68–203) μmol/h/10 cm (p>0·5) in omeprazole and ranitidine treated subjects respectively. Salivary and gastric bicarbonate contaminating the oesophagus accounted for 14% and 3%, respectively, of total oesophageal bicarbonate output.**

***Conclusions*—Bicarbonate secretory capacity of the human oesophagus is less than previously assumed, and the clinical relevance of intrinsic oesophageal bicarbonate for mucosal defence may be overestimated. As omeprazole and ranitidine did not affect bicarbonate secretion differently there was no evidence that omeprazole acts on bicarbonate secretory cells in the oesophageal mucosa.**

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Keywords: bicarbonate secretion, oesophagus, omeprazole, ranitidine.

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Bicarbonate secretion by the human oesophagus – supposed to originate in submucosal glands – has recently been implicated as a protective mechanism against damage from refluxed gastric acid.[1 2] However, reported secretory rates varied from 156 to 489 μmol/ h/10 cm, which clouds the interpretation that intrinsic oesophageal bicarbonate secretion is important for clearance of refluxed gastric acid compared with the salivary component of acid neutralisation. The findings of an incremental increase in oesophageal pH with repeat swallows[3 4] and stimulation of salivary bicarbonate secretion by oesophageal acidification, suggesting the existence of an oesophago-salivary reflex,[2 5] have previously established the importance of salivary bicarbonate. On the other hand, in vitro experiments suggest that intrinsic oesophageal bicarbonate secretion rates of about 160 μmol/h are capable of neutralising 1 ml of acid with a pH >2·0,[1] which seems representative of the residual volume present within the oesophageal lumen after an episode of reflux and bolus clearance by peristalsis.[3 4]

The proton pump inhibitor omeprazole has recently been shown to enhance duodenal mucosal bicarbonate secretion in healthy volunteers independent of its gastric acid inhibitory effect.[6] This increase in duodenal neutralising capacity is likely to add to the well documented superiority of omeprazole in duodenal ulcer healing over histamine H_2 receptor antagonists. In the treatment of gastro-oesophageal reflux disease omeprazole is again superior to histamine H_2 receptor antagonists.[7 8] It remains to be established whether omeprazole has a stimulatory effect of its own on oesophageal bicarbonate secretion, which might contribute to the therapeutic gain. We have, therefore, made an attempt to measure human oesophageal bicarbonate secretion more accurately to allow assessment of the in vivo effect of omeprazole on intrinsic oesophageal bicarbonate secretion in healthy volunteers.

Methods

SUBJECTS

Ten healthy volunteers (six men and four women, median age 26, range 20–42 years), with no past or current gastrointestinal or other medical diseases, were studied by a protocol approved by the Copenhagen and Frediriksberg ethics committee. Each participant gave written informed consent.

EXPERIMENTAL DESIGN

Nine of the subjects were studied twice after an overnight fast. Before perfusion the subjects were randomly pretreated with either omeprazole (60 mg/day for three days and 80 mg

intravenously one hour before perfusion) or ranitidine (600 mg/day for three days and 50 mg/h intravenously during perfusion). Firstly, a double lumen gastric tube (16 French, AN 10 Anderson Samplers Inc, Atlanta, GA, USA) was introduced nasally and placed in the distal antrum. The infusion port was located 8 cm proximal to the aspiration port. Secondly, a similar double lumen tube was introduced orally and placed in the distal oesophagus. The infusion port was located 10 cm orally to the aspiration ports, which were positioned 35 cm from the incisors (Figure). Experiments were performed with subjects in a semireclined position. The oesophagus was perfused (Ivac 560 Pump, NC Nielsen, Glostrup, Denmark) with isotonic saline (5 ml/min; pH 7·0) using [^{51}Cr]EDTA (10 μCi/l) as a non-absorbable marker. The stomach was similarly perfused with isotonic saline containing phenol red (50 mg/l; pH 7·0), at a constant rate of 2 ml/min (LKB 2115 Multiperpex Pump, Bromma, Sweden). During experiments, the subjects were instructed to avoid swallowing saliva, which was removed by buccal aspiration. After an initial 30 minute equilibration period all effluents were collected at 15 minute intervals for one hour (from the mouth, oesophagus, and stomach) by intermittent suction (Pump AB, Einar Egnell, Trollhättan, Sweden) for analysis.

ANALYTICAL PROCEDURES
The pH values of the aspirates were immediately measured by a glass electrode attached to a standard pH meter (PHM82, Radiometer, Copenhagen, Denmark), which was calibrated daily by standard buffer solutions at pH 4, 7, and 10.

Concentrations of bicarbonate in 100 μl aliquots of saliva and aspirates from the oesophagus and the stomach were determined in triplicate (Corning 965 Carbon Dioxide Analyzer, Corning Ltd., Halstead, UK). This method corresponds to the back titration method,[9] which has previously been shown to determine oesophageal bicarbonate secretion more accurately than the pH/pCO$_2$ method.[2] Before analysis the samples were gassed with CO$_2$ free nitrogen for five minutes to remove dissolved carbon dioxide. The Corning analyser was calibrated daily against 1·0 and 2·5 mM NaHCO$_3$. The between day variability, expressed as coefficient of variation, was 12% (n=20) and 6% (n=20) respectively.

To determine the contamination of the oesophageal test segment with saliva, amylase concentrations were measured in samples of saliva and aspirates from the oesophagus by an enzymatic, colorimetric method using malto-heptaoside (Merck, Darmstadt, Germany) as a substrate.

Activities of [^{51}Cr] and concentrations of phenol red were determined both in oesophageal and gastric aspirates and served as markers for recovery and contamination of the respective test segment. Activities of [^{51}Cr] were measured by gamma spectrometry. Concentrations of phenol red were measured spectrophotometrically at 560 nm after being made alkaline (pH=11) by a 4+1 dilution with a 0·5 M Na$_2$PO$_4$ solution.

VALIDATION STUDIES

In vitro bicarbonate studies
The accuracy of the Corning 965 carbon dioxide analyser for determination of bicarbonate concentrations was tested by performing 10 measurements on each of 0·5, 1·0, 2·5, 5·0, and 10·0 mM standard bicarbonate solutions. These results were compared with those obtained by using the original back titration method.

In vivo infusion of bicarbonate
In three of the subjects additional experiments were performed to determine the accuracy of the perfusion method for measurement of oesophageal bicarbonate secretion. After a 30 minute equilibration period, basal bicarbonate secretion was determined for 30 minutes as described above. During the next four 30 minute periods a 5 ml bolus containing 100, 250, 500, or 1000 μmol sodium bicarbonate was infused and total bicarbonate output was calculated. Subsequently, the amount of infused bicarbonate was calculated by subtracting basal bicarbonate secretion from total bicarbonate output during the periods of exogenous bicarbonate infusion.

Sputum amylase, HCO$_3^-$

^{51}Cr (5 ml/min)

10 cm

HCO$_3^-$, Amylase, phenol red, ^{51}Cr

Phenol red (2 ml/min)

10 cm
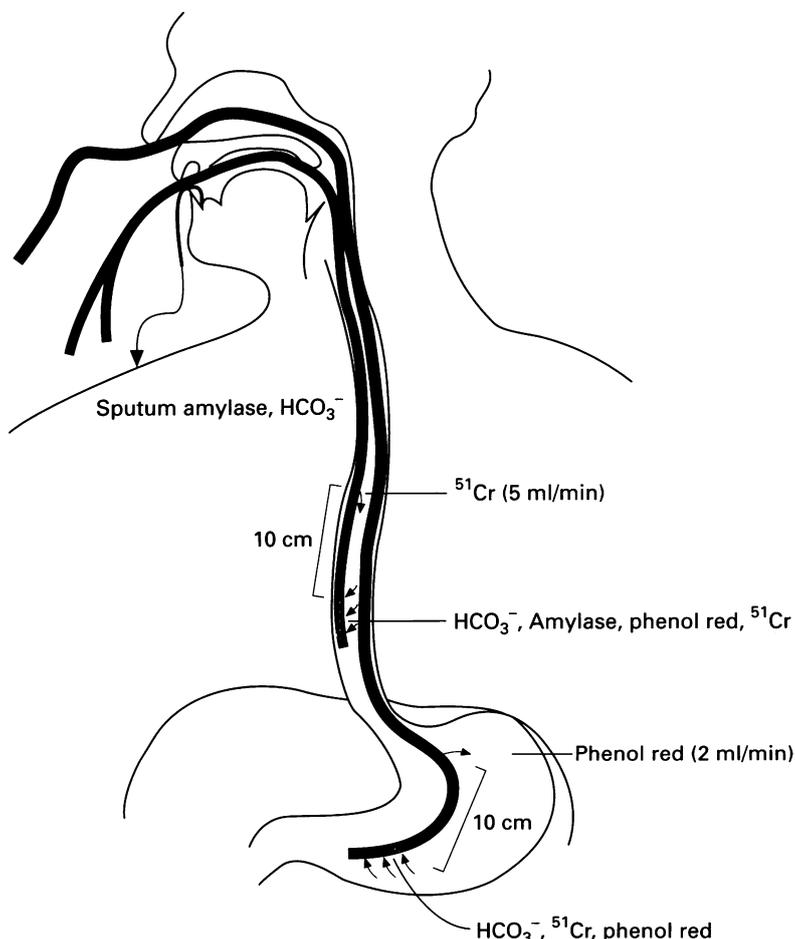
HCO$_3^-$, ^{51}Cr, phenol red

Diagram of tube position for measuring intrinsic oesophageal bicarbonate secretion and contamination by swallowed salivary and refluxed gastric bicarbonate in the human oesophagus.

CALCULATIONS AND STATISTICAL ANALYSES

Previously described formulas for a "steady state" perfusion system were used for calculation of oesophageal fluid output and bicarbonate secretion, in addition to contamination of the oesophageal test segment with salivary and gastric bicarbonate.[2][10] Rates of intrinsic oesophageal bicarbonate secretion were calculated by subtracting contaminating saliva and gastric bicarbonate from the total amount of bicarbonate in the oesophageal perfusate in individual 15 minute periods and given as means of the four 15 minute values obtained in each experiment.

The results were expressed as median values with 95% confidence intervals (95% CI). The Wilcoxon signed rank test and the Spearman rank order correlation test were used for statistical analyses.[11] All p values calculated were two tailed and the α level of significance was set at 0·05.

Results

VALIDATION OF METHOD

In vitro bicarbonate studies

Analysis of standard bicarbonate solutions (0·5, 1·0, 2·5, 5·0, 10·0 mM) by the Corning analyser showed a highly significant correlation ($r=0·99$; $p<0·001$) between measured and known concentrations. Also the results obtained by back titration showed a high degree of correlation with those obtained by the Corning analyser ($r=0·99$; $p<0·001$).

In vivo infusion of bicarbonate

The amounts of bicarbonate infused into the oesophagus were linearly correlated with the bicarbonate output adjusted for intrinsic oesophageal bicarbonate secretion ($r=0·96$; $p<0·005$). Also, the amounts of bicarbonate infused into the oesophagus during the first two 30 minute periods (100 and 250 μmol

sodium bicarbonate) corresponded with those secreted by the oesophageal segment.

Measurement of pH and marker recovery

There were no significant differences between omeprazole and ranitidine treated subjects in median values of oesophageal and gastric pH measurements, salivary and oesophageal amylase concentrations, or oesophageal and gastric recoveries of [^{51}Cr] and phenol red (Table I).

BASAL BICARBONATE SECRETION

The median rates of intrinsic oesophageal bicarbonate secretion, corrected for contaminating salivary and gastric bicarbonate, were 89 (33–150) and 121 (68–203) μmol/h/10 cm after pretreatment with omeprazole and ranitidine respectively ($p>0·5$).

The outputs of bicarbonate in saliva after omeprazole and ranitidine pretreatment were 493 (116–3552) and 1532 (1016–3967) μmol/h respectively ($p<0·05$). All oesophageal aspirates contained varying low concentrations of amylase, indicative of swallowed saliva. The median contents of salivary bicarbonate contaminating the oesophageal test segment were 22 (1–50) μmol/h in omeprazole experiments and 16 (13–26) μmol/h in ranitidine experiments ($p>0·5$), thus accounting for about 18% and 10%, respectively, of total oesophageal bicarbonate output.

The outputs of bicarbonate into the stomach after omeprazole and ranitidine pretreatment were 344 (259–828) and 307 (286–513) μmol/h ($p>0·5$) respectively. Reflux of gastric bicarbonate was not found in all oesophageal samples. The median contents of gastric bicarbonate refluxed into the oesophageal test segment were 3 (0–4) μmol/h in omeprazole experiments and 4 (2–80) μmol/h in ranitidine experiments ($p<0·02$), thus accounting for about 3% of total oesophageal bicarbonate output.

Table II presents the data obtained in individual subjects.

TABLE I *pH measurements and recoveries of phenol red and [^{51}Cr] in oesophagus and stomach, and amylase concentrations in saliva and oesophagus after pretreatment with omeprazole (OME) and ranitidine (RAN) (medians and 95% CI; n=9)*

	pH		[^{51}Cr] (%)		Phenol red (%)		Amylase (kU/l)	
	Oesophagus	Stomach	Oesophagus	Stomach	Oesophagus	Stomach	Saliva	Oesophagus
OME	6·8 (6·6–6·9)	6·8 (6·7–7·0)	93 (93–99)	19 (6–31)	0·3 (0–0·6)	73 (57–83)	108 (82–256)	0·4 (0·3–2·8)
RAN	6·7 (6·6–6·8)	6·9 (6·7–7·0)	91 (79–97)	19 (5–28)	0·6 (0–7·3)	69 (56–83)	132 (94–245)	0·5 (0·2–2·4)

TABLE II *Individual data in nine subjects indicating intrinsic oesophageal bicarbonate output and contamination by salivary bicarbonate and refluxed gastric bicarbonate after pretreatment with omeprazole and ranitidine*

	Omeprazole			Ranitidine		
No	Net oesophageal HCO₃ output (μmol/h/10 cm)	Salivary HCO₃ contamination (μmol/h)	Gastric HCO₃ contamination (μmol/h)	Net oesophageal HCO₃ output (μmol/h/10 cm)	Salivary HCO₃ contamination (μmol/h)	Gastric HCO₃ contamination (μmol/h)
1	77	1	1	38	16	0
2	135	10	4	68	14	80
3	79	46	0	127	21	4
4	25	1	0	88	8	2
5	150	22	12	91	13	351
6	150	164	3	121	23	11
7	122	50	1	129	26	4
8	33	25	3	284	16	4
9	89	6	3	203	89	8

Discussion

The results of this study support recent findings suggesting that human oesophagus has the ability to secrete bicarbonate into the lumen.[1][2] The origin of bicarbonate has been attributed to submucosal glands present in human oesophagus.[1][2] Rabbit oesophagus, which is devoid of submucosal glands, does not seem to secrete bicarbonate,[13] which emphasises the role of these glands in bicarbonate secretion by the oesophagus.

The functional relevance of intrinsic oesophageal bicarbonate secretion and other protective mechanisms, such as salivary bicarbonate and mucous secretion and oesophageal mucous secretion, motor activity, mucosal blood flow, and epithelial cell proliferation and resistance, is to maintain mucosal integrity after exposure to aggressive factors, such as acid and pepsin.[14] The importance of the low rates of oesophageal bicarbonate secretion for neutralisation of gastric acid seems, however, overestimated in the light of the part played by salivary bicarbonate and peristaltic movements. In the present study, the rates of bicarbonate secretion into saliva were about 10-fold higher than the output of intrinsic oesophageal bicarbonate. Similarly, other studies have clearly shown the importance of saliva for acid clearance in the oesophagus[3][4] and the capability of oesophageal peristalsis to reduce the bolus from a reflux period to less than 1 µl. Nevertheless, the median rates of oesophageal bicarbonate secretion found would still be sufficient to neutralise a volume <1 ml with a pH >2·5,[2] which is consistent with the previously noted non-salivary component of luminal acid clearance.[3][4][15] Furthermore, the oesophagus seems to have additional capacity for luminal acid clearance, as it has been shown that topical acidification stimulates bicarbonate secretion.[16]

Isolation of intestinal segments by occluding balloons for prevention of contaminating fluids from adjacent segments provides an attractive model for the in vivo study of ionic transport in the gastrointestinal tract. This approach has proved useful in the human duodenum and jejunum, but the application of two occluding balloons in the oesophagus, by using a commercially available, specially designed tube,[17] was unsuccessful in our hands in preliminary experiments. The subjects had great difficulty in tolerating the pressure from the inflated balloons and a free aspiration flow could not be sustained. As Meyers and Orlando[1] experienced similar difficulties, we chose to adopt the technique described by Brown et al,[2] which involves continuous perfusion of a 10 cm open segment of the distal oesophagus. This procedure did not result in larger contamination with salivary bicarbonate than in studies using occluded segments.

In the present investigation of nine healthy volunteers the median rates of intrinsic oesophageal bicarbonate secretion were 89 (25–150) and 121 (38–284) µmol/h/10 cm, respectively, after pretreatment with omeprazole and ranitidine. These results are similar to previous findings by Meyers and Orlando,[1] who reported a median rate of intrinsic oesophageal bicarbonate secretion in 10 healthy subjects of 156 (10–472) µmol/h/10 cm. Surprisingly, Brown et al[2][16] found median rates of 306 (96–510) and 489 (130–1050) µmol/h/10 cm, respectively, and the same low contamination by salivary and gastric bicarbonate, but these investigators achieved a much lower recovery in the perfused segment than that in the present study.

As reflux of acidic gastric contents to the distal oesophagus may neutralise the small amounts of secreted bicarbonate, suppression of gastric acid secretion is mandatory to obtain valid results of oesophageal bicarbonate secretion. In previous studies on human oesophageal bicarbonate secretion ranitidine was used for acid suppression.[1][2] We compared the effect of omeprazole on oesophageal bicarbonate secretion with that of ranitidine, because of recent findings that omeprazole enhances bicarbonate secretion in the duodenum.[6] Also the finding that high doses of ranitidine (about 2 g daily) are inferior to omeprazole in reducing the number of reflux episodes, despite equally significant reduction in gastric acid secretion,[18] suggests that factors other than considerable and prolonged acid inhibition[19][20] may contribute to the therapeutic effect of omeprazole. Nevertheless, the results of the present study do not support the hypothesis that omeprazole and ranitidine in equipotent doses act differently on oesophageal bicarbonate secretion. The theory that omeprazole may act directly on a bicarbonate transport mechanisms seems unlikely.

In conclusion, the present study supports the concept that intrinsic oesophageal bicarbonate secretion may add to the salivary component of acid neutralisation, but the secretory capacity of the mucosa lining the oesophageal wall seems lower than previously assumed. As omeprazole and ranitidine did not affect oesophageal bicarbonate secretion differently it is unlikely that omeprazole acts on bicarbonate secretory cells.

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