Effect of bismuth subcitrate and sucralfate on rat duodenal and human gastric bicarbonate secretion in vivo

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Abstract
Acid and alkali secretion have been examined together with prostaglandin E₂ production in response to two mucosal protective drugs, colloidal bismuth subcitrate and sucralfate. Doses of colloidal bismuth subcitrate in the therapeutic range (120 and 1200 mg) had no effect on alkali secretion or luminal PGE₂ output when perfused into the stomach of human volunteers. Similarly, in the anaesthetised rat, neither gastric acid nor duodenal alkali secretions were influenced by iv (12 mg/kg) or topical (120 mg/ml) administration of colloidal bismuth subcitrate. In contrast, perfusion of the human stomach with 1 g sucralfate stimulated bicarbonate output by 50%, a response which was unaffected by indomethacin (25 mg/h). A rise of 64% in gastric PGE₂ output after sucralfate was, however, prevented by indomethacin pretreatment. Alkali secretion by rat duodenum was also increased by sucralfate but the response depended on the basal secretory rate. Low basal secretors (<3 μmol) showed a 75% stimulation whereas rats with high basal secretory rates (>3 μmol) showed no significant response. All duodenal preparations regardless of basal secretory rate showed a stimulation of bicarbonate output with topical PGE₂. The results suggest that enhancement of gastroduodenal bicarbonate secretion may play a role in the protective action of sucralfate but is unlikely to explain mucosal protection by colloidal bismuth subcitrate.

Sucralfate and colloidal bismuth subcitrate (CBS) have both been shown to protect gastroduodenal mucosa against experimental damage⁶ and to heal peptic ulcers in man.⁷ Neither agent affects acid secretion⁶ and their mechanisms of ulcer healing and mucosal protection remain unclear.

Sucralfate, a basic aluminium salt of sucrose octasulphate, was originally proposed to act by adhering to damaged and ulcerated tissue thereby forming a physical barrier against injury by luminal acid, pepsin and bile salts.⁸,⁹ More recent studies indicate that this drug has additional actions on gastroduodenal mucosa which may contribute to its protective effect. For example, sucralfate has been reported to stimulate prostaglandin generation,¹⁰ bicarbonate secretion,¹¹ and mucus production¹² in laboratory animals.

Colloidal bismuth subcitrate precipitates in the acidic environment of the stomach which would encourage binding to the mucosal surface in a similar manner to sucralfate. In addition to the formation of a physical barrier, this adherence could also initiate an irritant reaction which would lead to the reported formation and release of endogenous prostaglandins.¹³,¹⁴ Clinical interest in colloidal bismuth subcitrate (De-Nol¹⁵, Gist-Brocades) has recently been aroused by reports of lowered duodenal ulcer relapse rates in comparison with H₂ receptor antagonists.¹⁶-²⁰ The reasons for this are unclear although the bactericidal action of bismuth against Campylobacter pylori is the currently favoured hypothesis.²¹

The present studies were designed to evaluate the actions of sucralfate and colloidal bismuth subcitrate on mucosal bicarbonate secretion in the rat duodenum and human stomach.

Methods

Animals
Experiments were performed using female Sprague-Dawley rats (200-220 g body weight) obtained from ICI Pharmaceuticals animal breeding unit. Rats were starved for 20-24 hours before use in cages with wide mesh bottoms to prevent coprophagy but allowed free access to drinking water.

Measurement of secretion
Duodenal bicarbonate secretion was measured as previously described.¹² Briefly, animals were anaesthetised with urethane 1.5 g/kg im. Body temperature was maintained at 37°C by means of a heated table and intrarectal thermistor. A 12 mm segment of the descending part of the duodenum, starting 5 mm distal to the outlet of the bile duct and about 2 cm from the pylorus, was cannulated in situ. The bile duct and pylorus were ligated. A femoral vein was cannulated to enable infusion of saline (1 ml/kg/h) to prevent dehydration and for administration of drugs. The duodenal segment was perfused with 4 ml of 0.15 M NaCl at 37°C which was circulated rapidly by a gas lift of 100% oxygen prewashed in Ba(OH)₂. The rate of alkalinisation was determined by continuous titration to pH 7.4 with 25 mM HCl made isotonic with NaCl. Titrant delivery was controlled automatically using standard pH-stat equipment (GK 2321C pH electrode, ABU 80, PHM 62, TTT 80, all Radiometer Copenhagen, Denmark).

For measurement of acid secretion, the stomach was perfused at 7 ml/min with saline through the oesophagus and fluid collected by an outlet tube in the pylorus. Secretion was
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stimulated with the H₂ agonist dimaprit (SK&F, Welwyn, UK) given as a 10 mg/kg bolus and 30 mg/kg/h sc infusion. Acid output was determined every 10 min for a total of two hours by titration of samples with 20 mM NaOH to pH 6.4.

Experimental design
After completion of surgery, the preparation was allowed to stabilise for about one hour. Alkalisation (µmol/cm²/h) was determined at 15 minute intervals and mean rates of secretion (SEM) compared before and after drug treatment by paired t test.

Basal alkali secretion was measured for 60 minutes then either sucralfate or bismuth subcitrate added to the luminal solution in 100 µl volumes to give a final concentration of 1 mg/ml for sucralfate and 11.7 or 117 mg/ml (10 and 100 µmol) for bismuth subcitrate. The effects of iv administration of colloidal bismuth subcitrate (11.7 and 117 mg/kg) were also determined in a separate series of experiments. In the case of sucralfate, drug addition induced transient fluctuations in the pH of the luminal perfusate. A 20 minute re-equilibration period was therefore allowed after drug addition before recommending measurement of alkali secretion for a further 60 min. The viability and sensitivity of individual preparations was assessed at the end of each experiment by recording maximal secretory response 45 min after adding 10 µg/ml prostaglandin E₂ (PGE₂) to the luminal solution.

Drugs
Sucralfate was obtained as an amorphous powder (Ayerst Laboratories, Andover, UK) and formulated as a saline suspension. Addition of 100 µl to the luminal solution was equivalent to adding 1 mg/ml of the aluminium salt of sucrose octasulphate.

Bismuth subcitrate was also obtained as an amorphous powder (Gist-Brocades, Delft, The Netherlands) and prepared as a concentrated solution in saline at room temperature so that addition of 100 µl produced a final concentration of 117 mg/ml.

PGE₂ (Sigma Chemicals, St Louis, MO) was dissolved in ethanol, stored at –20°C and added to the luminal solution in a volume of 30 µl to produce a final concentration of 10 µg/ml.

Human Studies
Subjects
Studies were performed in 20 male and six female healthy volunteers aged between 18 and 27 years who were taking no medication. Four groups of subjects received either 1 or 10 mg/ml bismuth subcitrate (both n=6), 8 mg/ml sucralfate (n=8), or sucralfate plus 0.21 mg/ml indomethacin (n=6).

Measurement of secretion
Gastric bicarbonate secretion by the intact human stomach was measured by an intubation method described previously. After an overnight fast the volunteers swallowed a multilumen polyethylene tube which was positioned by fluoroscopy with its tip in the second or third part of the duodenum. Ranitidine (150 mg orally one hour beforehand and 25 mg/h iv during the study) was given to maintain intragastric pH between 6 and 7. Saliva was continuously removed by suction which reduced salivary contamination to below 3% of measured gastric bicarbonate. After aspiration of resting gastric contents, the stomach and duodenum were perfused at 2 ml/min with saline containing 12.5 µCi/l of H₂-polyethylene glycol and C₂-polyethylene glycol respectively. Aspirates were collected by continuous suction at 10 minute intervals from the antrum and duodenum for measurement of marker concentration. Aliquots (1 ml) were frozen for later assay of PGE₂ concentration. In the middle of each sampling period a separate 1 ml aliquot was aspirated into a cooled syringe for immediate determination of pH and pCO₂.

Analytical methods
Concentrations of H₂-PEG and C₂-PEG in the samples were measured by liquid scintillation counting (LKB Wallace 2000 Beta Counter) with external quench correction. The pH and pCO₂ of the samples were measured using a Corning 170 Blood Gas Analyser. Prostaglandin E₂ concentrations were measured by radioimmunoassay after extraction into 2 ml diethyl ether, evaporation then resuspension into 1 ml radioimmunoassay buffer. Recovery experiments revealed a good correlation between amounts of PGE₂ added and measured (102 (15)% of that added, mean (SD), n=8) suggesting low cross-reactivity with potential interfering substances.

Experimental design
After an equilibration period of one hour, basal bicarbonate secretion was measured for 60 minutes. The gastric perfusate was then changed to H₂-PEG containing either sucralfate in suspension (8 mg/ml) or a bismuth subcitrate solution (1 or 10 mg/ml) and perfusion continued for a further hour. Total amounts perfused were equivalent to 1 g sucralfate and either 120 or 1200 mg bismuth subcitrate. In the third hour there was a return to the original perfusate.

To study the effects of cyclooxygenase inhibition on the response to sucralfate, the gastric perfusate was changed to H₂-PEG containing indomethacin (0.21 mg/ml) halfway through the first basal period. After 30 min the gastric perfusate was again changed to H₂-PEG containing indomethacin (0.21 mg/ml) plus sucralfate (8 mg/ml) for a further hour before returning to the original perfusate (H₂-PEG alone) in the third hour.

Drugs
Bismuth subcitrate was obtained as an amorphous powder and made up as a solution in H₂-PEG to give a final concentration of either 1 mg/ml or 10 mg/ml. Although colloidal bismuth
subcitrate possesses little buffering capacity, the suspension was titrated to pH 7-4 before administration. Sucralfate was obtained in suspension and diluted to produce a concentration of 8 mg/ml. This formulation was also titrated to pH 7-4 before use.

Indomethacin (Sigma Chemicals, Poole, UK) was prepared as an aqueous solution of concentration 0·21 mg/ml at pH 7-4.

Calculation of results
Gastric and duodenal volumes were calculated from marker dilution and perfusion rate. Duodenogastric reflux was calculated from the amount of ^3H-PEG appearing in the gastric aspirate. Free bicarbonate concentration was calculated using the Henderson-Hasselbalch equation and was added to that bicarbonate in the form of CO₂ (pCO₂×solubility constant of 0·031) in order to determine total bicarbonate concentration. Output was calculated as the product of volume and concentration corrected for refluxed bicarbonate. Similarly, prostaglandin E₂ output was the product of concentration and volume. All results are expressed as means (SEM) and differences assessed by paired t test.

Results
ANIMAL STUDIES

Bismuth subcitrate
Topical application of 10 and 100 µmol colloidal bismuth subcitrate had no effect on alkali secre-

tion by rat distal duodenum (Fig 1). The preparation responded to topical PGE₂ (10 µg/ml) which induced a peak stimulation of 110% from 6·60 (1·65) to 15·4 (2·63) µmol/cm/h (p<0·01, n=5).

Similarly, intravenous administration of 10 µmol/kg had no effect on alkalinisation while topical PGE₂ (10 µg/ml) increased secretory rate by 120% from 4·30 (0·60) to 9·0 (1·56) µmol/cm/h (p<0·01, n=6) (Fig 1). This dose of colloidal bismuth subcitrate was without effect on dimaprit stimulated gastric acid secretion in the rat over a two hour period (acid secretion=174 (14) µmol in controls and 182 (19) µmol in treated animals, p>0·1, n=10). When the higher dose of colloidal bismuth subcitrate (100 µmol/kg (117 g/kg)) was administered systemically, all the animals died.

Sucralfate
Sucralfate induced two distinct responses depending upon basal secretory rate. Topical application of sucralfate 1 mg/kg to rats with high rates of basal alkali secretion (>3 µmol/cm/h, n=4) had no effect on alkali secretion. Thus secretory rate during drug exposure (5·45 (1·30) µmol/cm/h) was similar to that during the basal period (5·55 (1·36)) although PGE₂ (10 µg/ml) stimulated secretion by 90% from 4·90 (1·17) to 9·30 (3·11) µmol/cm/h, p<0·05 (Fig 2).

In the animals with low basal alkali secretion (<3 µmol/cm/h, n=4) topical sucralfate produced a rise in alkali secretion of 175% from 1·20 (0·33) to 3·3 (0·97) µmol/cm/h, p<0·05. These preparations appeared more sensitive to PGE₂ which increased secretory rate by 460% over the initial basal value (from 1·20 (0·33) to 7·0 (1·85) µmol/cm/h, p<0·05).
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Human Studies

Bismuth subcitrate
Neither concentration of bismuth subcitrate influenced alkali secretion of luminal PGE₂ output by the human stomach (Table).

Sucralfate
During perfusion of the stomach with 1 g sucralfate over one hour there was a significant increase in alkali secretion from 494 (74) to 741 (88) μmol/h (n=8, p<0.01) which returned towards basal values when the drug containing suspension was replaced by PEG during the third hour (Fig 3). There was no change in bicarbonate originating from duodenogastric reflux during the three study periods. There was no significant change in PGE₂ output when the basal period (1099 (103) pmol/h) and sucralfate perfusion period (1136 (121) pmol/h) were compared (p>0.1). However a significant rise (p<0.05) in PGE₂ output was recorded during the final hour (1799 (205)). The stimulation in bicarbonate output induced by sucralfate was due to an increase in gastric fluid secretion with the volume rising from 66 (16) to 135 (33) ml/h during drug perfusion (Fig 4). There was no significant increase in bicarbonate concentration during exposure to sucralfate (3.09 (0.22) and 3.40 (0.27) mmol/l, p>0.1). The increase in PGE₂ output, however, was not a result of the increase in fluid secretion since the PGE₂ and volume response were clearly dissociated.

Indomethacin and sucralfate
Perfusion of the stomach with indomethacin alone (12.5 mg over 30 minutes) produced a fall in basal alkali secretion, although this reduction did not achieve statistical significance (696 (160) μmol/h basally and 384 (93) during indomethacin perfusion, n=6, p=0.058). A higher dose of indomethacin (25 mg) caused a similar, insignificant reduction in alkali output and had no influence on the stimulation of alkali secretion induced by 1 g sucralfate which increased from 384 (93) μmol/h to 782 (132) μmol/h (p<0.05) and then returned to basal values during the final hour (Fig 5).

Luminal PGE₂ output was lower during the period of indomethacin perfusion alone but this reduction failed to reach statistical significance (p=0.68). No further change in PGE₂ output occurred during or after perfusion of the stomach with indomethacin plus sucralfate (Fig 5), in contrast with the rise in luminal PGE₂ output observed during this final hour with sucralfate alone (Fig 3).

Discussion
Our results show quite different effects for two commonly used mucosal protective and ulcer healing agents. In the case of colloidal bismuth subcitrate we have been unable to show any effects on human gastric and rat duodenal bi-

Table: Gastric bicarbonate (μmol/l) and luminal PGE₂ (pmol/l) outputs during perfusion of the human stomach with either 1 or 10 mg/ml colloidal bismuth subcitrate (mean (SE), n=6 for each concentration). Colloidal bismuth subcitrate was perfused during the second hour only

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Output</th>
<th>1st hour</th>
<th>2nd hour</th>
<th>3rd hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/ml</td>
<td>HCO₃⁻</td>
<td>593 (118)</td>
<td>631 (163)</td>
<td>631 (124)</td>
</tr>
<tr>
<td></td>
<td>PGE₂</td>
<td>1005 (130)</td>
<td>1210 (165)</td>
<td>1106 (163)</td>
</tr>
<tr>
<td>10 mg/ml</td>
<td>HCO₃⁻</td>
<td>453 (114)</td>
<td>412 (86)</td>
<td>489 (108)</td>
</tr>
<tr>
<td></td>
<td>PGE₂</td>
<td>898 (136)</td>
<td>998 (110)</td>
<td>1060 (134)</td>
</tr>
</tbody>
</table>

Figure 3: Effect of sucralfate (1 g) on human gastric bicarbonate and luminal PGE₂ outputs (mean (SE), n=8, *p<0.01).

Figure 4: Effect of sucralfate on human gastric secretory volume and bicarbonate concentration (mean (SE), n=8, *p<0.05).
Our dissociation between stimulation of gastric bicarbonate generation by delayed carbonate secretion and mucosae by indomethacin pretreatment of amphibian gastric mucosa. A similar finding has been reported in respect of stimulation of mucus production by sucralfate. It is possible that sucralfate acts on the (prostaglandin-independent) Cl-/HCO₃⁻ exchange component of duodenal alkaline secretion. This contention is reinforced by the fact that sucralfate stimulates alkaline secretion in the stomach where anion exchange is thought to be the major mechanism of HCO₃ transport. If, in the group of rats with high basal alkaline secretion, CI/HCO₃⁻ exchange is operating maximally then an agent stimulating such exchange would be expected to show little or no effect on bicarbonate output whereas a agent stimulating electrogenic secretion such as PGE₂ would remain effective. Such an explanation is consistent with our results which show that high basal secretion in the duodenum is associated with a response to PGE₂ but not sucralfate while low basal secreting preparations responded to both sucralfate and PGE₂.

These observations raise the intriguing possibility that sucralfate may act in three separate ways. First, by adhering to ulcerated mucosa it may produce a physical barrier to luminal acid and pepsin. Second, stimulation of endogenous prostaglandin metabolism may enhance endogenous protective mechanisms that influence both ulcer healing and mucosal protection. Finally, sucralfate may directly stimulate the production of mucus gel and transport of bicarbonate by surface epithelial cells, possibly by a local irritant reaction. The latter effects would seem unlikely to play a role in ulcer healing but may be important in preventing damage to the intact epithelium.

It is possible that different components of sucralfate are responsible for these various actions. For example, sucrose may contribute to the ability of the complex to adhere to the mucosa and thus form a physical barrier, while aluminium can directly stimulate bicarbonate secretion. Further, the protective action of sucralfate on oesophageal mucosa has been proposed to result from the presence of sulphate. It thus seems likely that the protective action of sucralfate is multifactorial with different components activating various mechanisms of gastroduodenal mucosal defence.

Both sucralfate and colloidal bismuth subcitrate (De-Nol) are primarily ulcer healing agents. Stimulation of bicarbonate secretion by sucralfate would be expected to enhance preepithelial acid disposal in the stomach and duodenum. In theory, such a property could prevent ulcer recurrence after initial healing, alleviate NSAID induced gastric damage, and reduce stress ulceration in severely ill patients. It is difficult to envisage, however, what role this would have in accelerating chronic peptic ulcer healing. Indeed, the absence of a viable epithelium in the region of an ulcer implies that the diseased mucosa would be unable to respond to stimulants of bicarbonate secretion. Thus we
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The authors provided was a SERC (CASE) student. Expert technical assistance was provided by Linda Gibbons, Susan Hampton andangan Stanier.

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Gut 1990 31: 26-31
doi: 10.1136/gut.31.1.26