Effect of intragastric pH on mucosal protective action of sucralfate

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SUMMARY Acid intragastric pH is believed to be mandatory for mucosal protective action of sucralfate, but evidence for its efficacy at neutral pH is lacking. The effect of sucralfate on gastric mucosal erosions induced by oral administration of aspirin and bile acids at acidic pH of 1.5 and 3.9, and near neutral pH of 6.5 was investigated in 320 rats. The effect of sucralfate on the intragastric pH four hours after the ingestion of test solutions was also examined. The incidence and severity of mucosal erosions induced by aspirin and bile acids were lower in animals treated with sucralfate at acidic ($p < 0.001$) and near neutral ($p < 0.01$) intragastric pH. Mucosal protection was greater with ingestion of sucralfate 300 mg/kg and 200 mg/kg, than with 100 mg/kg. The intragastric pH was higher ($p < 0.001$) in sucralfate treated groups at pH 3.9 and 6.5. This study provides evidence that sucralfate protects against mucosal injury at near neutral, as well as at acidic pH.

The ulcer healing properties of sucralfate¹⁻³ and its ability to protect the gastric mucosa from acute experimental injury⁴⁻⁶ are related to its local actions⁶ rather than the inhibition of acid secretion. Its mode of action is, however, multifactorial in that its acute administration has been found to stimulate a range of mucosal defence factors including prostaglandins,⁷ mucus,⁸ and bicarbonate.⁹ In addition, however, the physiochemical properties of the drug allow it to bind to the mucosa,¹⁰⁻¹² and more selectively to ulcer sites,¹³ forming a barrier against the diffusion of acid, pepsin, and other noxious agents. Sucralfate may also deactivate pepsin^{14,15} and adsorb bile salts,^{16,17} but these effects have been thought to require an acid environment.^{10,11} For example, the binding of bile salts *in vitro* is considerably less under neutral as opposed to acid conditions.¹⁷ For these reasons the efficacy of sucralfate in the protection of the mucosa against damaging agents such as aspirin and bile acids, has been questioned under conditions where acid secretion is itself inhibited or absent.

Previous studies which have examined the mucosal protective effects of sucralfate against acute injury have in general been conducted under acid pH conditions. The present study has been designed to

determine whether sucralfate has mucosal protective properties in rats subjected to acute gastric injury with aspirin and bile salts at acidic and near neutral intragastric pHs. We also studied the effect of different doses of sucralfate on mucosal protection and the effects of this drug on the intragastric pH.

Methods

SOLUTIONS

For the experiments at pHs 1.5 and 3.9, ASA was administered at a dose of 105 mg/kg body weight and for experiments at pH 6.5 315 mg/kg was used. The bile acids taurodeoxycholic acid (TDCA), taurocholic acid (TCA), and glycocholic acid (GCA) were added at concentrations of 5 mmol/l for pylorus intact and 10 mmol/l for pylorus ligated rats. Sucralfate was used at doses of 100, 200, and 300 mg/kg. The vehicle for the control and test solutions contained 28 mmol/l d-glucose, 16.5 mmol/l sodium chloride, 0.25% carboxymethylcellulose, and 2.5% gum arabic. Solutions at pHs 1.5, 3.9, and 6.5 were buffered using 0.2 mol/l citrate buffer, 0.2 mol/l acetate buffer, and 0.67 mol/l phosphate buffer respectively.

ANIMALS

Experiments were carried out on Sprague-Dawley (n=320) rats weighing between 150 and 250 g. The

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animals were kept in wire-floored cages throughout to limit coprophagia. They were starved for 24 hours before the experiment but allowed free access to water. Groups of animals ($n=10-13$) received either the control solution or test solutions of aspirin alone, and in combination with bile salts, with and without the addition of sucralfate. In order to minimise intra-gastric pH change of the test solution throughout the course of the experiment, rats who received test solutions buffered to pH 6.5, had their pylorus ligated before administration of the solutions. The abdomen was opened under ether anaesthesia, the pylorus ligated, the abdominal wound sutured and rats were allowed to recover from anaesthesia. In rats which received the test solutions buffered to pHs. 1.5 and 3.9, the pylorus was maintained intact. Both groups of pylorus ligated and pylorus intact rats were weighed, and a constant volume per body weight (13.3 ml/kg) of solution was administered by per oral intubation. Four hours later the animals were killed by an overdose of ether. The stomachs were removed, opened along the greater curvature, rinsed with water and the mucosal surface wiped with a cotton bud. The glandular portion was then examined for mucosal erosions by another investigator without previous knowledge of the rat groupings. Rats were scored positive or negative for the occurrence of mucosal erosions. The severity of the lesions was determined: the lesions induced were usually linear and their length and width were measured. Each lesion was scored according to the product of their diameter in both directions. A lesion measured less than 1×1 mm was scored as 1, 2×1 mm as 2; 3×1 mm as 3; 2×2 as 4 and so on. The sum score of all lesions was expressed as the total lesion score for each rat. In each experiment, a similar number of rats from each group were studied at any one time, on separate days. A similar number of controlled rats received the vehicle solution only.

MEASUREMENT OF INTRAGASTRIC pH AND PLASMA SALICYLATE

The pH of intragastric luminal contents in experiments at pHs 3.9 and 6.5 was measured using a miniature pH electrode¹⁸ (model MI-508, Micro-electrodes Incorporated, New Hampshire, USA). Through an incision made along the stomach wall, the electrode was introduced and placed in contact with the gastric luminal contents. Blood samples for measurement of salicylic acid were obtained from the inferior vena cava immediately before the animals were killed. Salicylic acid was measured using a standard spectrophotometric method.¹⁹

STATISTICAL ANALYSIS

Statistical comparisons were made using the

Student's *t* test, or when the data were non-parametric, the Mann-Whitney test.

Results

EFFECT OF SUCRALFATE ADMINISTERED AT pH 3.9 ON MUCOSAL PROTECTION

Figure 1 shows the incidence and severity of mucosal erosions induced by exposing groups of rats ($n=10$) to aspirin (105 mg/kg) in the absence and presence of sucralfate (SF) at dosages of 100, 200, and 300 mg/kg. Rats which received sucralfate showed significantly less mucosal erosions at the three doses used ($p<0.005$, $p<0.001$, and $p<0.001$).

Figure 2 shows similarly that the presence of sucralfate resulted in significant protection from ASA, when its damaging effect was potentiated ($p<0.005$) by the simultaneous administration of TDCA (5 mmol/l). The protection from damage by ASA alone or when combined with TDCA, became more pronounced with larger doses of sucralfate. As sucralfate at the dose of 300 mg/kg appeared to produce the greatest mucosal protection, all the following experiments were done using this dose. The effectiveness of sucralfate in protecting against lesions induced by ASA and TDCA, was also demonstrable using 5 mmol/l of GCA and TCA ($p<0.001$), (Fig. 3).

In these, and the following experiments, a similar number of animals receiving only the control solution showed no detectable mucosal lesions.

EFFECT OF SUCRALFATE ADMINISTERED AT pH 1.5 ON MUCOSAL PROTECTION

Groups of rats ($n=10-13$) were exposed to ASA (105 mg/kg) alone or together with each of the bile acids GCA, TCA, and TDCA (5 mmol/l) with and without sucralfate. The addition of bile salts to ASA resulted

Fig. 1 Effect of different doses on sucralfate (SF) at pH 3.9 on mucosal erosions induced by ASA.

Fig. 2 Effect of different doses of sucralfate (SF) at pH 3.9 on mucosal erosions induced by ASA in combination with bile salt (TDCA).

in higher erosion scores than ASA alone. In this respect TCA was more injurious than TDCA which in turn was more harmful than GCA (Table 1).

Figure 4 shows that sucralfate protects the gastric mucosa against damage by ASA alone, and ASA in combination with each of the bile acids GCA, TDCA, and TCA ($p<0.001$).

Fig. 3 Effect of sucralfate (300 mg/kg) administered at pH 3.9 on mucosal erosions induced by ASA in combination with bile salts (TCA & GCA).

Table 1 Effect of bile salts (5 mmol/l) on the severity of lesions induced by ASA (105 mg/kg) at pH 1.5

Groups	Erosion scores (mean (SE))	Significance* (v ASA)
ASA	6.6 (0.8)	—
ASA+GCA	10.5 (1.7)	$p<0.05$
ASA+TDCA	15.5 (1.5)	$p<0.001$
ASA+TCA	18.6 (0.9)	$p<0.001$

*Significance by Mann-Whitney test.

Table 2 Effect of sucralfate (300 mg/kg) on pH of intragastric contents

Groups	pH of test solution	Intragastric pH (mean SE)	Significance*
ASA+bile salts		1.51 (0.07)	
ASA+bile salts+SF	3.9	2.47 (0.09)	$p<0.001$
ASA+bile salts		6.0 (0.06)	
ASA+bile salts+SF	6.5	6.4 (0.07)	$p<0.001$

*Significance by Mann-Whitney test.

EFFECT OF SUCRALFATE ADMINISTERED AT pH 6.5 ON MUCOSAL PROTECTION

The dosages of ASA and bile salts were increased to 315 mg/kg and 10 mmol/l respectively, in order to induce sufficient lesions at this pH. The results (Fig. 5) show that at pH 6.5 sucralfate protects against lesions induced by ASA in combination with each of the bile salts GCA, TDCA, and TCA ($p<0.01$).

EFFECT OF SUCRALFATE ON INTRAGASTRIC pH

To simplify the results of intragastric pH, no subdivision of groups into individual bile salts was made because this had no statistical effect on the results. The intragastric pH was significantly higher in animals that received sucralfate at pHs 6.5 and 3.9 (Table 2).

EFFECT OF SUCRALFATE ON PLASMA SALICYLATE CONCENTRATION

The plasma salicylate concentration was measured

Table 3 Effect of sucralfate on blood salicylate concentration

Groups	pH of test solution	Plasma salicylate (µg/l) (mean SE)	Significance*
ASA (105 mg/kg)+bile salts		226 (4.0)	
ASA (105 mg/kg)+bile salts+SF (300 mg/kg)	3.9	224 (4.5)	NS
ASA (315 mg/kg)+bile salts		160 (12)	
ASA (315 mg/kg)+bile salts+SF (300 mg/kg)	6.5	140 (15)	NS

*Significance by Mann-Whitney test.

Fig. 4 Effect of sucralfate (300 mg/kg) administered at pH 1.5 on mucosal erosions induced by ASA alone and in combination with bile salts (GCA, TCA, and TDCA).

four hours after ingestion of solutions. As the effect of each of the bile salts on the absorption of sucralfate was not of interest, no subdivision of groups into individual bile salts was made. There was no significant difference in salicylate levels at pHs 3.9, or 6.5 with and without sucralfate (Table 3).

Discussion

The present study has shown for the first time that sucralfate protects against acute mucosal injury induced by aspirin and bile acids at near neutral pH as well as at acidic pHs. The protective effect of sucralfate against acute experimental injury has been previously demonstrated in rats; sucralfate was administered 15–30 minutes before aspirin,⁷ and the bile acid TCA,⁴ both acidified to pH 1.5. By contrast, we administered sucralfate simultaneously with aspirin and bile acids, at pHs above and below their dissociation constants (pKa). Sucralfate administered at an acidic pH and at doses smaller than those used by other investigators, resulted four hours later in marked mucosal protection against injury induced by aspirin alone and when combined with different bile acids. Our results indicate that pretreatment with sucralfate is not necessary for the induction of mucosal protection. Administration of sucralfate in combination with the damaging agent may prolong or even improve the extent of protection, however, because mucosal protection is observed to be maximal within 60 minutes and to disappear almost completely after four hours of pretreatment with sucralfate.⁷ As mucosal injury becomes obvious within 10 minutes of ingestion of

Fig. 5 Effect of sucralfate (300 mg/kg) administered at pH 6.5 on mucosal erosions induced by ASA in combination with bile salts (GCA, TCA, and TDCA).

aspirin.^{20,21} initiation of this injury and protection by sucralfate would be expected to have taken effect when the intragastric pH in experiments conducted at pHs 3-9 and 1-5 were still different. In separate experiments, with rats killed at different intervals, we have observed that when aspirin is administered at pH 3-9, the pH of the intragastric contents is maintained around this level for about an hour before the intragastric pH is gradually reduced to pH 1-5, four hours later.

To conduct the experiments at near neutral pH, we had to ligate the pylorus during laparotomy. This procedure may have added a stress factor in this group but did not contribute to the induction of mucosal erosions, as the control animals did not show any erosions. Without pylorus ligation we were unable to induce mucosal erosions at pH 6-5, and to maintain a high intragastric pH during the course of the experiment. Although this can be achieved by a continuous infusion of a buffer in the pylorus-intact animal, rapid emptying of the gastric contents results, thus preventing adequate contact of the test solution with the gastric mucosa. Alternatively a high intragastric pH can be obtained by inhibiting gastric acid secretion using a hydrogen ion blocking agent. With such an intervention, however, we would have been unable to study the effect of sucralfate on the intragastric pH, and may have introduced a potential interference with mucosal protection from another drug.

In the acid milieu of gastric juice, the sucralfate molecule dissociates releasing aluminium and sucrose octasulphate ions.^{1,10} The polyvalent anions on the sucrose octasulphate groups interact with the cationic charges of mucosal proteins and possibly mucus,⁷ forming a protective barrier over the mucosal surface.¹¹⁻¹³ The degree of protection afforded at any specific location may be dependent on saturation of binding sites on mucosal proteins. This may partly explain the greater mucosal protection observed in this study, when the dose of sucralfate was increased from 100 mg to 200 mg/kg than that obtained by the increment from 200 to 300 mg/kg.

It appears that sucralfate retains its physico-chemical characteristics at a pH near to neutral. Sucralfate is insoluble in water and alcohol but becomes viscous and partially soluble at an alkaline as well as an acidic pH,¹⁰ and we had no difficulty in maintaining the drug in suspension at pH 6-5. As part of its characteristic local effects, however, sucralfate exerts an influence on other mucosal defense factors. Recent studies have demonstrated a role for the drug in the stimulation of mucosal prostaglandin synthesis,⁸ mucus production,²² and the prevention of peptic degradation of mucus.²³ Interestingly,

sucralfate promotes mucosal regeneration¹⁸ and it is possible that this may be related to an effect on mucosal blood flow.

The normal acid secretory capacity of the stomach resulted in a reduction of the intragastric pH, to the usual pH of 1-5, in animals who received aspirin and bile acids at pH 3-9. Nevertheless, the rats treated with sucralfate showed a higher intragastric pH than those which did not receive the drug, in the pylorus intact and pylorus ligated groups. There are two possible explanations for this observation. First, the sucralfate molecule has the ability to buffer hydrogen ion through the dissociation of aluminium hydroxide.^{11,24} This is thought to be a weak effect in human gastric juice²⁵ but could be more important in the rat model because of the lower volume of gastric juice and higher relative dose of sucralfate used. Alternatively sucralfate may increase bicarbonate secretion and there are data derived from the isolated amphibian mucosa²⁶ and man⁹ to support this hypothesis. An increased production of bicarbonate could in turn result from either the secretion of mucus^{8,27} or enhanced postaglandin production by sucralfate.^{28,29}

Bile acids are incriminated in the pathogenesis of mucosal damage.^{30,31} As with aspirin, bile acids produce mucosal damage by increasing mucosal permeability and decreasing mucosal bicarbonate secretion.³² In this way bile acids potentiate the damaging action of aspirin.^{33,34} This effect has been clearly shown in this study. At pHs below their dissociation constant (PKa), aspirin (PKa=3.5) and the bile acids (TDCA and TCA, PKa=1.9 and GCA PKa=3.8) become less ionised³⁵ and more damaging to the mucosa.³⁶ The converse is also true which explains why doubling the bile salt concentration and trebling the ASA dose, was required to induce mucosal damage at pH 6-5. Although these bile acids are more ionised at this pH, *in vitro* studies have shown that the bile acids used in this study are adsorbed by sucralfate to a much lesser extent at a neutral than at an acidic pH.^{10,17} The adsorption of bile salts by sucralfate is most probably related to the affinity of the trivalent aluminium cations for the anionic groups of the bile salts. Such binding is markedly affected by the nature of the steroid and amino acid moiety of the conjugated bile acid, but pH is noted to have no effect *per se* on the binding of bile acids with aluminium hydroxide *in vitro*.³⁷ Our results show that sucralfate protects the gastric mucosa from bile acid induced injury, at pHs above and below their pKa values. This suggests that either the bile acid binding characteristics of sucralfate are independent of pH, or that other protective factors play a more important role in its mechanism of action.

The binding of sucralfate to aspirin is not considered to be a factor in its protective effect against

aspirin induced injury. *In vitro* studies have shown that aspirin is minimally adsorbed by sucralfate¹⁷ (1.4 mg/g sucralfate). Furthermore it has been observed that sucralfate does not influence salicylate absorption in man.⁵ Our finding of a similar serum salicylate concentration four hours after ingestion of aspirin, in the presence and absence of sucralfate, accords with a lack of a major binding effect on aspirin by sucralfate. At higher pHs salicylate is mainly ionised and so is poorly absorbed. This, and the prevention of small bowel absorption due to pyloric ligation, explains the lower plasma salicylate levels at pH 6.5.

We have provided evidence that sucralfate retains its protective action against mucosal injury, in the rat model, at near neutral intragastric pH. The dosages that we have used are considerably higher on a weight for weight basis than those used in man. It is, however, well established that the dose of sucralfate required to produce mucosal protection at an acid pH is much higher in the rat model^{4,6,7} than in man.^{5,29} The demonstration of protection at a near neutral intragastric pH, therefore, also deserves further assessment in humans. There is some recent clinical data to suggest that concomitant administration of an H₂ blocker does not reduce the efficacy of sucralfate to heal duodenal ulcers.³⁸ It is interesting to speculate as to whether sucralfate might also provide protection against alkaline agents, such as bile. Our study suggests that further clinical trials to confirm these findings at human dosage levels would be of considerable interest in the future.

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