Stimulation of amphibian gastroduodenal bicarbonate secretion by sucralfate and aluminium: role of local prostaglandin metabolism

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SUMMARY The present studies were designed to explore the possible mode of protective and ulcer healing actions of sucralfate by examining its effect on gastroduodenal bicarbonate secretion by isolated amphibian mucosa. Luminal sucralfate (0.5 g/l) significantly increased bicarbonate secretion by fundic and antral mucosa without influencing transmucosal potential difference. Significant stimulation of duodenal bicarbonate secretion occurred only at 1.0 g/l without change in potential difference. Aluminium, a component of sucralfate, produced similar increases in bicarbonate secretion, while the sucrose and sulphate components were without effect. Pretreatment of mucosae with the cyclooxygenase inhibitor, indomethacin (10^{-5} M) did not abolish the secretory response to sucralfate or aluminium. The results suggest that stimulation of gastroduodenal bicarbonate secretion, possibly by the aluminium moiety of sucralfate, may play a role in its protective and ulcer healing actions.

Sucralfate is a basic aluminium salt of sucrose sulphate and has been shown to protect gastric mucosa from experimental damage,¹ prevent bleeding from stress ulceration,² and heal peptic ulcers.^{3,4} The drug was initially thought to act by adhering to ulcerated tissue and forming a physical barrier to injury by acid, pepsin, and bile salts.⁵⁻⁷ Recent evidence has suggested that sucralfate may have a direct protective action on the gastroduodenal mucosa, possibly mediated by changes in local prostaglandin E_2 metabolism.⁸ Such an action may be shared by other compounds containing aluminium such as antacids, raising the possibility that this component may be vital to the protective action of sucralfate.⁹ The current series of experiments were designed to further explore the mechanism of mucosal protection by sucralfate by studying its action and that of aluminium on gastroduodenal bicarbonate secretion, an important component of the 'mucus-bicarbonate' barrier.¹⁰

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Methods

FROGS

The studies on gastroduodenal bicarbonate secretion were performed on gastric and duodenal mucosal obtained from either *Rana temporaria* (Xenopus, UK) or *Rana catesbeiana* (St Croix Biologicals, Minneapolis, Minnesota, USA). All animals were kept at 21°C and used within three weeks of purchase.

STUDIES ON GASTRIC MUCOSA

The method used to measure electrical and secretory activity of gastric mucosa has been previously described in detail.¹¹ Briefly, gastric mucosa was dissected free from its muscularis externa and mounted as a membrane (surface area 1.8 cm) between two halves of a perspex chamber. Each surface was bathed with 20 ml solution circulated by means of gas lifts. The unbuffered luminal side solution was gassed with 100% oxygen and the buffered serosal side solution with a mixture of 95% oxygen and 5% carbon dioxide. The pH of the

luminal side solution was maintained at pH 7.40 by the addition of 5 mM hydrochloric acid from a pH stat system (ABU 13 and TT2, Radiometer, Copenhagen, Denmark).

Spontaneous output of acid by fundic mucosa was inhibited by adding 10^{-3} M cimetidine to the serosal side solution. Antral mucosa spontaneously secreted alkali. Transmucosal potential difference was measured by means of matched electrodes (Russel Electrodes, Fife, Scotland) and continually recorded while electrical resistance was determined from the fall in recorded potential difference produced by passing a 30 μ A current through the tissue via silver/silver chloride electrodes. The mucosal and serosal solutions were maintained at 20°C by water jackets perfused by a Haake G circulator (Karlsruhe, W. Germany).

STUDIES ON DUODENAL MUCOSA

The duodenal mucosa was stripped of muscularis externa and mounted as an intact tube on glass cannulae as previously described.¹² The luminal surface was bathed with 10 ml unbuffered solution, circulated by 100% oxygen gas lift and maintained at pH 7.40 using pH stat titration as described above. The serosal surface was bathed with 120 ml buffered solution gassed with a mixture of 95% oxygen and 5% carbon dioxide. Transmucosal potential difference was measured and recorded as described above and the bathing solutions maintained at 20°C.

BATHING SOLUTIONS

The serosal solution bathing gastric and duodenal mucosae contained Na^+ , 102.4 mM; K^+ , 4.0 mM; Ca^{2+} , 1.8 mM; Mg^{2+} , 0.8 mM; Cl^- , 91.4 mM; HCO_3^- , 17.8 mM; H_2PO_4^- , 0.8 mM; SO_4^{2-} , 0.8 mM; and glucose 2 mM (Osmolarity=220 mosmol and pH=7.20). The luminal solution differed in that HCO_3^- and H_2PO_4^- were replaced by mannitol (11.3 mM) to maintain the osmolarity. Titrant acid was identical to luminal solution except for the addition of 5×10^{-3} M hydrochloric acid.

DRUGS AND CHEMICALS

Sucralfate was obtained as the amorphous powder (Ayerst Laboratories, Hampshire, England) and prepared at room temperature as a suspension in saline so that additions of 200 μ l to bathing solutions produced final concentrations in the range of 0.1 to 1 g/l. Although sucralfate possessed little buffering capacity, the suspensions were titrated to pH 7.40 before addition to the chamber solutions. The pH of such suspensions remained stable and additions to gassed luminal solution in the absence of tissue did not alter the pH.

Indomethacin (Sigma Chemicals Ltd, England)

Fig. 1 Effect of luminal sucralfate (0.5 g/l) on alkalinisation and transmucosal PD of fundic mucosa ($n=4$, mean (SE), * $p<0.05$).

was prepared as a stock solution and added to the serosal side solution to produce a concentration range between 10^{-6} and 10^{-4} M.

Aluminium, in the form of aluminium potassium sulphate and aluminium acetate (Sigma Chemicals Ltd, England), was also prepared as stock solutions (pH 7.40) which did not disturb the pH equilibrium of the luminal solution, in the concentrations used. Potassium sulphate and sucrose (Sigma Chemicals Ltd) were prepared in a similar manner. Sucrose octasulphate (Ayerst Laboratories, Hampshire, England) was prepared to produce final luminal concentrations of 0.5 and 1.0 g/l.

STATISTICAL ANALYSIS

Secretory rates before and after the addition of drugs to the mucosa were compared by a paired t test. Values are expressed as mean (SE).

Fig. 2 Effect of luminal sucralfate (0.5 g/l) on alkanalisation and transmucosal PD of antral mucosa ($n=4$, mean (SE), * (SE), * $p<0.05$).

Results

In studies on *Rana catesbeiana*, sucralfate (0.5 g/l) increased the rate of alkaline secretion by fundic mucosa from 0.23 (0.08) to 0.68 (0.10) $\mu\text{mol}/\text{cm}^2/\text{h}$ ($p<0.005$, $n=4$) and antral mucosa from 0.29 (0.10) to 0.75 (0.10) $\mu\text{mol}/\text{cm}^2/\text{h}$ ($p<0.05$, $n=4$) when added to the luminal solution. The transmucosal potential difference and electrical resistance across these tissues were not altered (Figs 1, 2). In separate experiments, gastric mucosa from *Rana temporaria* exhibited a linear dose response to sucralfate in the concentration range 0.125–0.5 g/l (Fig. 3). The increased rate of secretion was not transient but persisted during the period of observation (Figs 1, 2).

Fig. 3 Dose response curves to sucralfate for duodenal (D) and gastric mucosa (G). Each point represents mean (SE) change in secretion ($n=4$ for G, $n=6$ for D).

These concentrations of sucralfate, however, did not influence duodenal bicarbonate secretion.

At a higher concentration of 1 g/l, sucralfate significantly increased duodenal alkali secretion (*Rana catesbeiana*) from 1.16 (0.30) to 1.69 (0.30) $\mu\text{mol}/\text{h}$ ($p<0.05$, $n=6$) without altering transmucosal potential difference (Fig. 4). In dose response studies, a linear relationship between rate of secretion and concentration used was observed, although compared with the gastric studies the curve was shifted to the right (Fig. 3). Addition of sucralfate (0.5 to 1.0 g/l) to the serosal solutions of gastric or duodenal mucosa did not alter the rate of alkali secretion (results not shown).

STUDIES OF COMPONENTS OF SUCRALFATE

In *Rana catesbeiana*, addition of $3 \times 10^{-3}\text{M}$ aluminium potassium sulphate (equivalent to the total aluminium content of 0.05 g/l sucralfate), to the luminal solution, increased alkali secretion by fundic (0.79 (0.30) to 2.04 (0.50) $\mu\text{mol}/\text{cm}^2/\text{h}$, $p<0.05$, $n=4$) and duodenal mucosa (1.29 (0.50) to 2.83 (0.60) $\mu\text{mol}/\text{h}$, $p<0.05$, $n=6$) without altering transmucosal potential difference (Figs 5, 6). Using gastric mucosa from *Rana temporaria*, the addition of aluminium acetate ($3 \times 10^{-3}\text{M}$) caused a similar stimulation of alkali secretion (0.11 (0.01) to 0.42 (0.04) $\mu\text{mol}/\text{cm}^2/\text{h}$, $n=6$, $p<0.05$).

Addition of sucrose or potassium sulphate (sucrose and sulphate concentrations equivalent to those found in 0.5 g/l sucralfate) to the luminal solution of gastric mucosa from *Rana temporaria* failed to alter alkali secretion or transmucosal potential difference. Furthermore addition of sucrose octasulphate to the luminal solution failed to significantly increase alkanalisation in concentrations of 0.5 g/l (0.44 (0.1) to 0.51 (0.1) $\mu\text{mol}/\text{cm}^2/\text{h}$, $n=7$, p :NS) and 1 g/l (0.42 (0.1) to 0.43 (0.1) $\mu\text{mol}/\text{cm}^2/\text{h}$).

In separate experiments, addition of sucralfate (0.5 to 1.0 g/l) to a chamber containing an inert

Fig. 4 Effect of luminal sucralfate (1 g/l) on alkalinisation and transmucosal PD of duodenal mucosa ($n=6$, mean $\pm$ SE), * $p<0.05$).

membrane (latex rubber) instead of viable mucosa did not result in titrable alkalinity, confirming that sucralfate did not disturb the pH stability of luminal solution.

MECHANISMS OF ALKALI SECRETION BY SUCRALFATE

Because the increase in gastroduodenal alkali secretion was not accompanied by changes in transmucosal potential difference, the secretory response is unlikely to result from mucosal damage, increasing passive bicarbonate diffusion, or electrogenic transport of bicarbonate across epithelial cell membrane. To confirm that the secretory response is dependant on a cellular transport mechanism, the effect of the metabolic inhibitor dinitrophenol (10^{-4} M) on the sucralfate effect was studied. Addition of dinitrophenol to gastric mucosa from *Rana temporaria* (serosal side solution) reduced basal alkali secretion from 0.28 (0.05) to 0.13 (0.02) $\mu\text{mol}/\text{cm}^2/\text{h}$, $p<0.05$, $n=4$) and transmucosal potential difference from 14.2 (1.8) to 3.9 (0.5) mV ($p<0.05$, $n=4$). Subsequent addition of sucralfate (0.5 g/l) to the luminal solution failed to increase the rate of alkalinisation (0.13 (0.03) to 0.12 (0.02) $\mu\text{mol}/\text{cm}^2/\text{h}$, $n=4$, p :NS).

ROLE OF ENDOGENOUS PROSTAGLANDINS IN THE RESPONSE TO SUCRALFATE AND ALUMINIUM

At concentrations of 10^{-6} M and 10^{-5} M, serosal side application of indomethacin did not prevent the

Fig. 5 Effect of luminal aluminium (3×10^{-4} M) on alkalinisation by fundic (F) and antral (A) mucosa ($n=4$, mean $\pm$ SE), * $p<0.05$).

increase in gastric alkali secretion (*Rana temporaria* mucosa) produced by 0.5 g/l sucralfate (0.17 (0.07) to 1.00 (0.06) $\mu\text{mol}/\text{cm}^2/\text{h}$, $n=6$, $p<0.05$ with 10^{-5} M indomethacin) or duodenal alkali secretion (*Rana catesbeiana* mucosa) produced by 1.0 g/l sucralfate (0.77 (0.08) to 2.08 (0.17) $\mu\text{mol}/\text{h}$, $n=6$, $p<0.05$ with 10^{-5} M indomethacin).

The response to 3×10^{-3} M aluminium potassium sulphate was also not affected by 10^{-6} and 10^{-5} M indomethacin (gastric: 0.10 (0.01) to 0.48 (0.20) $\mu\text{mol}/\text{cm}^2/\text{h}$, $n=6$, $p<0.05$; duodenal: 1.13 (0.03) to 1.76 (0.10) $\mu\text{mol}/\text{h}$, $n=6$, $p<0.05$; both series with 10^{-5} M indomethacin). At the high concentration of 10^{-4} M, indomethacin did abolish the alkali response to sucralfate by gastric and duodenal mucosa, but did not influence the aluminium responses. (Results not shown.)

Discussion

The results show that sucralfate and aluminium salts are capable of increasing alkali secretion from gastroduodenal mucosa by a prostaglandin independent mechanism. The secretory responses to sucralfate and aluminium were not associated with any alteration in transmucosal potential difference or electrical resistance (gastric mucosa only) implying that the transport of bicarbonate was neither electrogenic nor

Fig. 6 Effect of luminal aluminium (3×10^{-3} M) on alkalinisation by duodenal mucosa ($n=6$, mean (SE), * $p < 0.05$).

a result of increased passive diffusion across damaged epithelium. In support of a transcellular transport process dependant on oxidative metabolism was the observation that sucralfate stimulated alkali secretion was prevented by pretreatment of mucosa with dinitrophenol. Although not definitively proved, it seems likely that sucralfate and aluminium stimulate bicarbonate secretion by an electroneutral ion exchange mechanism, such as $\text{Cl}^-/\text{HCO}_3^-$ exchange. Such an exchange mechanism has been shown to account for the bulk of basal gastric bicarbonate secretion and may be activated in duodenal mucosa by encephalins, gastric inhibitory polypeptide and glucagon.¹¹⁻¹³

The finding that aluminium salts stimulate gastroduodenal bicarbonate secretion by a similar mechanism to sucralfate suggests that the aluminium component of sucralfate may be responsible for its action on alkali secretion. In solution, sucralfate undergoes dissociation with the release of aluminium, some of which may be in the form of complex ions.¹⁴ In the current study, the concentration of aluminium used was comparable with that present from sucralfate but probably exceeded the aluminium that dissociated from sucralfate in solution. Nevertheless, the other components of sucralfate, sucrose sulphate and sucrose octasulphate, did not influence gastroduodenal alkalinization in quantities comparable with those found in the drug suggesting that it is the aluminium content of sucralfate which is important. This contrasts with the mucosal protection afforded by sucralfate on oesophageal epithelium against acid injury which appears dependant on sucrose octasulphate.¹⁵ As oesophageal mucosa does not secrete bicarbonate in

significant quantities, however, it is unlikely that the protective effect of the drug in this tissue would be by the same mechanism.

There is evidence that sucralfate's action on gastroduodenal defence may be mediated by increased production of locally formed protective prostaglandins. Because exogenous prostaglandins of the E_2 and F_2 series have been shown to stimulate gastroduodenal bicarbonate secretion and suppression of endogenous prostanoid synthesis leads to its inhibition,¹⁶ it seemed possible that the action of sucralfate (and aluminium) could be mediated by enhanced prostaglandin formation. A number of early observations were not compatible with this hypothesis, however. First, stimulation of duodenal bicarbonate secretion by exogenous prostaglandins occurs by an electrogenic mechanism and is therefore accompanied by an increase in the transmucosal potential difference.¹² Second, the potency of prostaglandins on duodenal mucosa is normally greater than on gastric mucosa, the magnitude of the secretory response to applied prostaglandins being much greater.¹³ In the sucralfate experiments, the converse was observed with the duodenal alkali response being smaller than the gastric. The indomethacin experiments provided further evidence that the secretory response to sucralfate was prostaglandin independent. In concentrations believed to abolish cyclooxygenase activity (10^{-6} M), indomethacin failed to prevent the alkali response to sucralfate and aluminium by gastroduodenal mucosa. Therefore, although other protective mechanisms may be influenced by sucralfate's or aluminium's action on local prostanoid formation, there is no evidence that the bicarbonate response is dependent on such a mechanism. The same conclusion also seems to apply to stimulation of mucus production by sucralfate.¹⁷

The action of sucralfate and aluminium on gastroduodenal alkali secretion would serve to enhance the protection afforded by the 'mucus-bicarbonate' barrier. Because sucralfate has been shown to influence other components of mucosal defence¹⁷ it seems likely that its protective action may be mediated by a number of different mechanisms. The relative importance of each defence mechanism activated and whether effects on certain zones would protect against a specific noxious agent are issues which remain unanswered. The finding that aluminium *per se* is a stimulant of gastroduodenal bicarbonate secretion is also of interest in that aluminium containing antacids have been shown to protect mucosa by effects independent of acid neutralisation and bile salt binding.¹⁸ It is therefore conceivable that enhancement of the 'mucus-bicarbonate' barrier plays a role in this protective action of aluminium containing antacids.

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