J. Pharm. Pharmacol. 1985, 37: 776–780
Received May 16, 1985

The influence of native porcine gastric mucus gel on hydrogen ion diffusion: the effect of potentially ulcerogenic agents

N. C. TURNER, G. P. MARTIN* AND C. MARRIOTT

Department of Pharmacy, Brighton Polytechnic, Moulsecoomb, Brighton, BN2 4GJ, UK

Experiments were carried out to investigate the ability of native gastric mucus gels to retard hydrogen ion diffusion. Mucus held between two polycarbonate membrane filters in a diffusion cell, separating equimolar solutions of NaCl and HCl, significantly reduced the rate of hydrogen ion diffusion and increased the time for the mean hydrogen ion front to traverse the mucus compartment (lag time) when compared to an unstirred layer of saline ($P < 0.01$). *N*-Acetylcysteine, sodium taurodeoxycholate and acetylsalicylic acid significantly increased the diffusion rate ($P < 0.025$); the lag time was significantly reduced by *N*-acetylcysteine ($P < 0.001$). In addition mucus gels were found to have buffering capacity at a pH > 2. These observations suggest that native gastric mucus gels can retard hydrogen ion diffusion and that this retardation of diffusion is reduced by agents which are potentially damaging to the gastric mucosa.

The major organic secretion of the mucosal epithelium is gel mucus (Davenport 1977) and it has often been proposed that one of the functions of the superficial layer of mucus overlying the gastric epithelium, is protection of the underlying membrane from acid proteolysis. Several theoretical mechanisms of protection can be suggested such as the creation of an unstirred water layer next to the epithelium, the retardation of the transport of solute ions and molecules into and out of the bulk phase and buffering of the luminal acidity. However, whilst the mechanism of the cytoprotection is unresolved, it is clear that the permeability of the gel mucus to hydrogen ions and pepsin is a critical determinant of its role in mucosal protection.

Although Allen (1981) has reported that gastric mucus is an impermeable barrier to pepsin diffusion, the hypothesis that gastric mucus provides a barrier to hydrogen ion diffusion has largely been disregarded and it has been suggested that the rate of diffusion of the ions through mucus gels would not be much slower than that through an unstirred layer of similar thickness (Heatley 1959; Allen 1981; Moody & Zalewsky 1981). However, recent investigations have suggested that mucus glycoprotein gels do, in fact, retard the diffusion of hydrogen ions (Williams & Turnberg 1980; Pfeiffer 1981) decreasing the diffusion coefficient some four-fold when compared with hydrogen ion diffusion through unstirred saline (Williams & Turnberg 1980). This reduction in hydrogen ion diffusion rate could result in the creation of a proton gradient across the mucus gel. Although it has been suggested that it would have little impact as a time barrier (Thomson 1981; Grossman 1981), Vadgama & Alberti (1983) have demonstrated that mucus does present a significant and selective barrier to proton diffusion when compared with Na^+ and K^+ diffusion at a pH > 4.

Additionally it has been suggested that a further protective function of the mucus layer on the gastric epithelium would be to reduce the concentration of xenobiotics presented to the epithelial membrane by retarding their diffusion from the lumen. However the effect of potentially injurious agents on proton diffusion through mucus gels is unknown. It was the purpose of these experiments to re-examine the ability of native gastric mucus to function as a diffusion barrier to proton movement and to investigate the effects of agents which are potentially damaging to the gastric epithelium on hydrogen ion mobility.

METHODS

Diffusion studies

Experiments were carried out using native porcine gastric mucus collected, by gentle scraping, from the stomachs of freshly slaughtered pigs (*Suis scrofa domestica*). The mucus was pooled, divided into samples, frozen and stored at −32 °C until required.

Samples of mucus weighing 0·5 ± 0·02 g (n = 40) were sandwiched between two 0·4 μm polycarbonate membrane filters (Nuclepore, Pleasanton, USA), separated by a hollow spacer 1 mm thick and of 3 cm^2

* Correspondence.

cross sectional area. This mucus layer was clamped between two glass compartments of equal volume and suspended in a water bath maintained at 4 °C (Fig. 1). 100 ml of a 154 mM sodium chloride solution pre-cooled to 4 °C was placed into the receptor compartment (B) and the experiment initiated by the introduction of 100 ml of 154 mM hydrochloric acid (at 4 °C) into the donor compartment (A). Both proton donor and receptor compartments were stirred continuously. The pH of the saline in compartment B was monitored over 30 min using a dual glass electrode coupled to a pH meter (Cleriprobe 310D, Uniprobe Ltd, Cardiff) linked to a chart recorder.

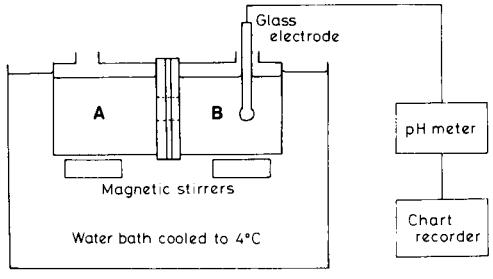

FIG. 1. Diagrammatic representation of the cell used to measure hydrogen ion diffusion through native porcine gastric mucus.

Hydrogen ion diffusion through mucus was compared with that through a saline layer of the same dimensions. The effect of added agents on hydrogen ion diffusion through mucus was investigated by dialysing the mucus sample in Visking sacs (molecular weight retention 10 000D) overnight against 154 mM saline containing the appropriate concentration of drug. The agents investigated were acetylsalicyclic acid (Thornton & Ross, Huddersfield), *N*-acetylcysteine (BDH Chemicals Ltd, Poole), sodium taurodeoxycholate (Sigma Chemical Co. Ltd, Poole) and ethanol. Control experiments were carried out in a similar manner except that the mucus was dialysed using 154 mM saline alone. Any changes in the volume of the gel after dialysis were found to be negligible (1·93 ± 0·7% w/w).

All experiments and dialyses were carried out at 4 °C to minimize any possible enzymatic degradation of the gel by pepsin, which may have been present in the crude gel removed from the stomachs.

The results of the diffusion studies are expressed as changes in hydrogen ion concentration with time. The diffusion rate (μmol min^{-1}) was calculated from the slope of the linear part of the diffusion curve and the lag time by extrapolation to the initial hydrogen ion concentration of the saline solution in chamber B. The results were analysed for statistical significance using the Mann-Witney U-test for unpaired observations (Siegel 1956).

Buffering capacity of gastric mucus

Samples of native gastric mucus (0·53 ± 0·012 g, n = 11), pre-treated with saline as described in the diffusion studies and homogenized in 154 mM saline to give a final volume of 5 ml, were titrated against 1 M HCl using an auto-burette (Radiometer, Copenhagen, Denmark) coupled to a chart recorder. Control experiments were carried out by titration of 5 ml samples of saline alone. Buffering capacity of the gel within the pH range 1·5 to 6·0 was calculated as the titre of saline + mucus minus that for saline alone.

RESULTS

Diffusion studies

The rate of diffusion of hydrogen ions across an unstirred layer of saline held in the diffusion cell was rapid (9·33 ± 1·93 μmol min^{-1}) and the time required for the mean hydrogen ion front to traverse the 1 mm distance (the lag time) was 1·9 ± 0·3 min. Native porcine gastric mucus significantly reduced the rate of hydrogen ion diffusion to 1·808 ± 0·15 μmol min^{-1} ($P < 0·001$) and in addition, the lag time for the appearance of acid in compartment B was significantly increased (9·88 ± 0·48 min, $P < 0·01$) (Fig. 2; Table 1).

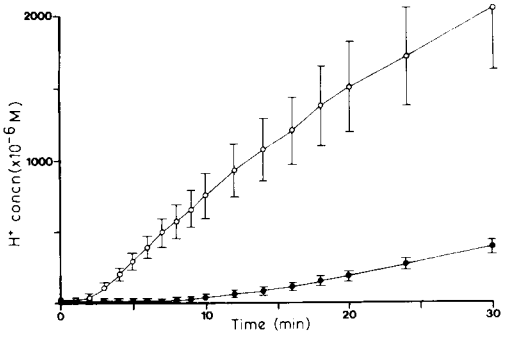

FIG. 2. Diffusion of hydrogen ions through a layer of porcine native gastric mucus 1 mm thick and 3 cm^2 cross-sectional area, (●) and through a layer of saline of the same dimensions (○). Each point is the mean ± s.e.m. of at least 6 observations.

The effects of pretreatment of the mucus gels with acetylsalicylic acid (ASA) (20 mM, adjusted to pH 6), sodium taurodeoxycholate (STDC) (20 mM), ethanol (ETOH) (20% v/v) and *N*-acetylcysteine (NAC) (100 mM) are shown in Fig. 3 and are summarized in Table 1. Preliminary studies involving NAC showed that diffusion of the free agent out of

Table 1. Effect of pretreatment with added agents on H^+ diffusion through native porcine gastric mucus gels 1 mm thick and of 3 cm^2 cross-sectional area.

	Diffusion rate (μmol min^{-1})	P	Lag time (min)	P	n
Saline control	9·330 ± 1·93		1·90 ± 0·3		6
Mucus control	1·808 ± 0·15	<0·001[1]	9·88 ± 0·48	<0·001[1]	10
Mucus pretreated with:					
Sodium taurodeoxycholate (20 mM)	2·558 ± 0·21	<0·01[2]	9·63 ± 0·39	>0·05	5
Acetylsalicylic acid (20 mM)	2·498 ± 0·216	<0·025[2]	8·46 ± 0·71	>0·05	7
Acetylcysteine (100 mM)	2·557 ± 0·267	<0·01[2]	2·94 ± 0·45	<0·001[2]	6
Ethanol (20% v/v)	1·435 ± 0·06	<0·05[2]	14·58 ± 1·22	<0·05[2]	6

Values represent means ± s.e.m. All experiments were carried out at 4 °C.
[1] Significant difference compared to saline control. [2] Significant difference compared to mucus control.
The level of significance was calculated using the Mann-Witney U-test.

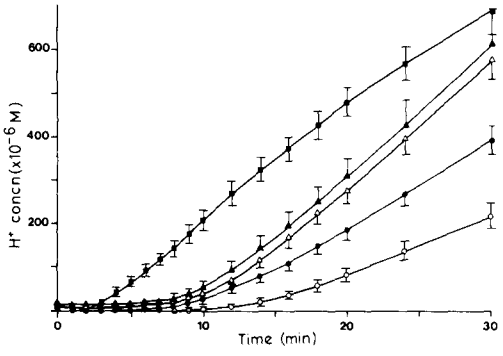

FIG. 3. Diffusion of hydrogen ions through a layer of porcine native gastric mucus 1 mm thick and 3 cm^2 cross-sectional area (●) and through layers of mucus which had been pretreated with 20 mM sodium taurodeoxycholate (△), 20 mM acetylsalicylic acid (▲), 100 mM *N*-acetylcysteine (■) or 20% v/v ethanol (○). Each point is the mean ± s.e.m. of at least 5 observations.

the gel resulted in a marked lowering of the resting pH of the saline in compartment B and therefore the treated gels were dialysed against saline, at 4 °C in order to remove the excess drug, for 1 h before the diffusion study.

ASA, STDC and NAC all increased the rate of hydrogen ion diffusion through the gel significantly ($P < 0·025$) whereas the rate of diffusion was significantly reduced by ETOH ($P < 0·05$). The time taken for the mean hydrogen ion front to traverse the gel (lag time) was significantly reduced by NAC ($P < 0·001$) and was increased by ETOH ($P < 0·01$), whereas STDC and ASA had no effect.

Buffering capacity

Fig. 4 shows the buffering capacity of 0·5 g samples of native gastric mucus gels over the range pH 6 to pH 1·5. A peak in buffering capacity was found at pH 2·5–4·0 and the total capacity was 14 ± 0·8 μmol HCl/0·5 g (7 ± 0·35 mmol $litre^{-1}$ pH $unit^{-1}$) assuming the density of the crude gel was 1 g ml^{-1}. There was no buffering capacity below pH 2.

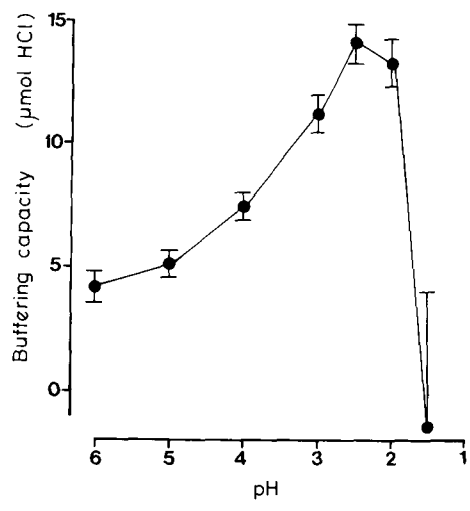

FIG. 4. Buffering capacity (μmol HCl) of samples of native porcine-gastric mucus over the range pH 1·5–6·0. Each sample of mucus gel was homogenized in 154 mM saline to give a final volume of 5 ml and titrated to pH 1·5 with 1 M HCl. The buffering capacity was calculated as the titre of mucus in saline minus that for saline alone. Each point is the mean ± s.e.m. of at least 11 observations.

DISCUSSION

The findings of the present study are in agreement with those of both Williams & Turnberg (1980) and Pfeiffer (1981) in demonstrating that mucus gels retard the diffusion of hydrogen ions approximately 5-fold when compared with diffusion through a layer of unstirred saline of similar thickness. Whilst these experiments do not enable elucidation of the

mechanism by which this reduction in diffusion rate occurs at least one hypothesis is available: the structure of gastric mucus is such that at the glycoprotein concentrations present in the gel it produces a dense gel matrix (Allen et al 1976) holding an unstirred water layer within its interstices, thus limiting ionic transfer. Furthermore, the hydrophilic nature of the oligosaccharide units of the glycoprotein macromolecule might result in the water in these regions being held in a more structured form rendering it largely unavailable for diffusion. Transient interaction of protons with charged moieties on the glycoprotein, particularly sialic acid and sulphate may also be involved in retardation of proton movement by electrostatic effects reducing ion mobility.

The functional significance of the barrier which native gastric mucus gels present to proton diffusion is uncertain since it has been shown that the thickness of the mucus layer adherent to the gastric mucosa, as measured in-vitro, is only approximately one tenth of that used in the present experiments and ranges from 70 μm in the rat up to 200 μm in man (Kerss et al 1982). It is possible, however, that the effective thickness of the unstirred water layer may be much greater and surface pH gradients of 700 μm have been reported on the rat stomach in-vivo (Allen et al 1983) although such an extended unstirred layer might be disrupted or eliminated by shear forces created during periods of increased gastric motility.

The significant increase in lag time for the arrival of acid in the receptor compartment when mucus, as opposed to saline, was included in the central spacer was in part attributable to the buffering capacity of the gel (Fig. 4). Pfeiffer (1981) discounted the effect of buffering on the diffusion of hydrogen ions through a commercially available mucus, although it should be appreciated that first the mucus employed in that investigation has been shown to be severely degraded by the extraction process (Marriott & Kellaway 1976) and second that a reinterpretation of the data obtained suggests that considerable buffering existed (Lucas 1984). Under normal physiological conditions the pH of the stomach rarely falls below pH 2 (Allen et al 1983) and hence the buffering capacity measured in this study could help maintain the pH gradient through the gel from lumen to epithelium (Ross et al 1982). Mucus being continually secreted by the mucosa to replace that lost to the lumen coupled with bicarbonate secretion (Allen et al 1983; Garner & Flemstrom 1978) at an appropriate rate (Vadgama & Alberti 1983) maintains the mucus at such a pH where buffering capacity is evident. This mechanism may provide the selective barrier to proton diffusion described by Vadgama & Alberti (1983). At a Luminal pH approaching 1, the more rapid diffusion of proton ions through the gel, as a result of the increased concentration gradient, causes the pH within the gel layer to fall rapidly (Ross et al 1982) concomitantly eliminating any buffering activity.

Pretreatment of the mucus gel with STDC, ASA and NAC produced similar increases in diffusion rate in each case (Table 1). However, although the direct effect of ASA on the physical structure of mucus gels is unknown, STDC and NAC have both been claimed to be mucolytic (Martin et al 1978; Martin et al 1980), albeit with differing modes of action. NAC is capable of disrupting the disulphide bonds which are purported to link the glycoprotein subunits to produce the polymeric molecule. This compound not only increases the diffusion rate but also reduces the lag time. In contrast STDC, which cannot reduce disulphide bonds to produce subunits, only affects the diffusion rate; the mucolytic activity of STDC is thought to be due to its effect on hydrogen and hydrophobic bonds. It would thus appear that ASA is acting by a similar mechanism to STDC and it is reported that salicyclic acid derivatives are capable of disrupting hydrogen bonds (Elworthy et al 1968). It therefore appears that it is necessary to reduce the mucus glycoprotein to subunits before any marked reduction in lag time can occur and that this effect is associated with a reduced gel barrier. Obviously ASA and STDC do not reduce the gel structure sufficiently to affect the lag time but they must affect the hydrogen ion bonding capacity sufficiently to allow an increased diffusion rate, perhaps via an increase in the cross-sectional area of free water available for diffusion. STDC, NAC and ASA have all been reported to reduce the ability of the mucus gel adherent to the rat fundic mucosa to maintain a pH gradient in-vivo (Ross et al 1981) and whilst this may result from their reported effects on mucus and bicarbonate secretion (Allen et al 1983; Rees et al 1981) an increase in hydrogen ion diffusion rate through the gel to levels exceeding the rate of bicarbonate secretion by the epithelium may also be involved.

The reduction in hydrogen ion diffusion following pretreatment of the gel with ethanol probably results from precipitation of protein within the gel further reducing the cross-sectional area of free water available for diffusion and suggests that gastric damage elicited by this agent in-vivo is unrelated to changes in mucus structure.

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