Gastric Mucosal Blood Flow in Misoprostol Pretreated Aspirin-induced Ulceration

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To determine whether topical misoprostol (a synthetic PGE_1 analog) pretreatment will increase or prevent a decrease in gastric mucosal blood flow (GMBF) during topical aspirin administration, we studied focal GMBF simultaneously by hydrogen gas clearance in a split canine gastric chamber model with one side as control. In the test chamber, immediately after topical misoprostol, there was a transient and significant increase (18%) in GMBF (55.71 ± 7.80 to 65.84 ± 6.12 mL/min/100 g; $p < 0.05$). After 15 minutes, GMBF returned to premisoprostol levels and then showed a graded drop throughout the aspirin and postaspirin periods. No grossly visible mucosal lesions were observed. In the control chamber, mucosal lesions were observed 45 minutes after aspirin administration accompanied by a graded drop in GMBF throughout the experiments. Misoprostol neither produced a sustained increase in GMBF nor prevented the subsequent reduction in GMBF induced by aspirin. Therefore, maintenance of GMBF may not be important in cytoprotection by misoprostol. The sustained nonparietal secretion induced by this synthetic PGE_1 analog may be important in gastric cytoprotection.

THE PRECISE MECHANISM(S) of gastric cytoprotection by prostaglandins (PG) is not known. The circulatory hypothesis proposes that the cytoprotective action is mediated through an increase in gastric mucosal blood flow (GMBF).[1-7] This hypothesis has, however, been faced with problems including (1) the results of studies of the effects of PG on GMBF have been inconsistent[3]; (2) though a number of the A, E, and I PG increase GMBF,[3,8-10] $PGF_{2\alpha}$, also cytoprotective,[11,12] is a known vasoconstrictor[8,13-16]; and (3) 16,16-dimethyl PGE_2 has been reported to prevent ulcer formation in the absence of arterial perfusion in isolated canine gastric mucosa,[17] in amphibian gastric mucosa *in vitro*,[18] and in gastric cell cultures in rats.[19,20]

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Misoprostol is a synthetic PGE_1 analog that has been reported to have both gastric antisecretory and cytoprotective properties.[21-30] There have been few reports in the literature on the effects of misoprostol on gastric blood flow. While some investigators have reported an increase in total gastric blood flow[2] and gastric mucosal blood volume,[31] others have reported either a decrease in total GMBF[23] or no effect.[27] We designed this study to elucidate further the circulatory hypothesis by a controlled investigation of focal GMBF changes using the hydrogen gas clearance (HGC) technique before, during, and after misoprostol pretreatment of aspirin-induced mucosal injury in a canine chambered gastric segment model. Specifically, the aim of this study was to test the hypothesis that pretreatment of the gastric mucosa with an antisecretory dose of misoprostol would increase or maintain gastric mucosal blood flow during aspirin injury. Second, the fluid and ionic fluxes were measured to determine any interrelationship between GMBF, fluid/ionic fluxes, and gastric cytoprotection.

Materials and Methods

Surgical Preparation

Four adult mongrel dogs of either sex, weighing 20–30 kg, were anesthetized with sodium pentobarbital (25 mg/kg) after a 24-hour fast (only water *ad libitum*). The dogs were intubated and maintained on a Harvard respirator (Harvard Apparatus, South Natick, MA) throughout each experiment. A hind leg vein was cannulated with a polyethylene catheter (PE 200) for infusion of 0.9% sodium chloride for the maintenance of hydration and a stable blood pressure. Arterial blood pressure was directly monitored by a saline-filled polyethylene cath-

eter (PE 200) in the left femoral artery via a Statham Gould P 23 Db transducer (Gould Statham Instruments Inc., Hato Ray, Puerto Rico). Following a midline laparotomy and splenectomy, a split chambered segment model of the gastric corpus with an isolated vascular pedicle was prepared according to the method of Moody and Durbin.[32] This model permits the use of one side of the chamber as test, the other as control and provides gastric mucosal surface of 17 cm^2 in each side. We have shown that electrode-mucosa contact is not a problem in this experimental model.[33] Body temperature and the temperature of the preparation were maintained by an electric blanket and infrared lamps. The preparation was allowed to stabilize for 60 minutes after surgery.

Experimental Design

Focal GMBF measurements were performed once every 15-minutes by two HGC electrodes placed in contact with the mucosa in each lumen of the chamber. One electrode was located in the upper and the other in the lower quadrants of each lumen throughout the experiments. Once the electrodes had been located at the beginning of each experiment there was no relocation throughout the experiments. Bathing solutions (8 mL) were instilled into and recovered from each lumen every 15-minutes as follows:

Test chamber. (1) basal periods (60 min): 100 mmol/L HCl + 50 mmol/L NaCl; (2) misoprostol periods (30 min): 100 mmol/L HCl + 50 mmol/L NaCl + 1 mL misoprostol (200 μg); (3) aspirin periods (60 min): 100 mmol/L HCl + 50 mmol/L NaCl + 20 mmol/L aspirin; and (4) postaspirin periods (120 min): same as for the basal periods.

Control chamber. Solutions instilled were the same as for the test chamber except in (2) where instead of misoprostol, 1 mL of vehicle (0.2 mL absolute ethanol + 0.8 mL phosphate buffer) was added to HCl and NaCl. Laboratory assays were performed on the recovered bathing solutions to determine the changes in volume, pH, sodium, and hydrogen ion concentrations. The pH of the bathing solutions and hydrogen ion concentration (titratable acid) were measured using a pH meter (Radiometer Copenhagen, Bach-Simpson Ltd., London, Ontario, Canada). Titration of the recovered solutions were performed to pH 2, the pH of the instilled solutions, with 0.1 N NaOH. Sodium ion concentration was measured with a Nova 1 Na^+/K^+ analyzer (Nova Biomedical, Newton, MA). The difference between the product of concentration and volume for the instilled and recovered solutions per 15 minute periods are the net fluxes expressed for sodium and hydrogen ions in the results below.

Misoprostol Preparation

Misoprostol was received as neat chemical in dry ice (G.D. Searle and Co. Inc., Skokie, IL). It was dissolved in isotonic 20% ethanol-containing phosphate buffer (pH 7.4). The misoprostol stock-solution was stored in vials at below −20 C when not in use and allowed to thaw to room temperature before use.

Hydrogen Gas Clearance

This was performed by standard technique as described previously.[33,34] The four HGC electrodes (Unique Medical Co. Ltd., Tokyo) with the four reference Ag/AgCl skin electrodes (Red Dot, 3M Canada Inc., London, Ontario, Canada) were connected to a Beckman multichannel recorder (Beckman Type R Dynograph, Beckman Instruments Inc., Schiller Park, IL) to obtain a permanent record of the measurements.

Histology

Following the termination of each experiment the mucosa and submucosa of tissue from both chambers were quickly dissected from the underlying muscularis and immersed in 10% neutral buffered formalin. The mucosa-submucosa preparation was then spread out and pinned flat on a cork slab within the fixative. After 24 hours fixation the tissue was Swiss-rolled and stored in 70% ethanol. Three random slices from each roll were resected and processed through to either paraffin and stained with hematoxylin and eosin or embedded in glycol methacrylate and stained with methylene blue-basic fuchsin. The slides were coded and examined blindly. Several morphologic parameters were noted and/or recorded for each sample including mucosal height, number and spatial relationship of gastric glands, integrity and continuity of the epithelial sheet, and presence of inflammatory cells in the lamina propria, as well as the appearance of mucosal capillaries. The sections were organized into groups based on the morphologic analysis before the code was broken. Comparison of the groupings permitted evaluation of consistent morphologic features within and between each group.

Data Analysis

All data are expressed as mean ± SE. GMBF data are expressed in mL/min/100 g of tissue. Statistical analysis for significance at the 5% level was performed using either the paired Student's t test or ANOVA, and linear regression analysis. Because there is a significant difference in GMBF between the upper and lower quadrants of the gastric chamber model, in comparing flow values between the test and control chambers, they were paired upper *versus* upper and lower *versus* lower in the t-tests.

Results

Gross and Microscopic Appearance

In the test chamber, there was gross mucosal swelling within 5 minutes of topical application of 200 μg of misoprostol, a feature that persisted throughout the experimental period. No grossly visible lesions were observed either during or after aspirin administration. Mucosal integrity was maintained throughout the experiment except for an obvious increase in the amount of mucus adherent to the luminal surface. Histologic evaluation of the misoprostol-treated tissue revealed a swollen lamina propria giving the gastric glands a widely spaced appearance and increased mucosal height. There was no increase in cellularity of the mucosa. Vascular channels were easily identifiable, dilated but empty of any cellular components (Fig. 1).

In the control chamber, multiple focal punctate lesions appeared on the mucosa 45 minutes after aspirin administration. Mucosal swelling did not occur within this chamber. Typical aspirin-induced superficial erosions could be identified in localized areas (Fig. 2). Although the majority of the tissue exhibited a normal morphology, there was frequent observation of a widened zone of lamina propria just beneath the surface epithelium (Fig. 3).

Gastric Mucosal Blood Flow

Resting focal GMBF measured by the four HGC electrodes consecutively for 1 hour revealed a positional effect on blood flow in the four quadrants of the double-lumen chamber. In both test and control chambers, there was a highly significant difference between the

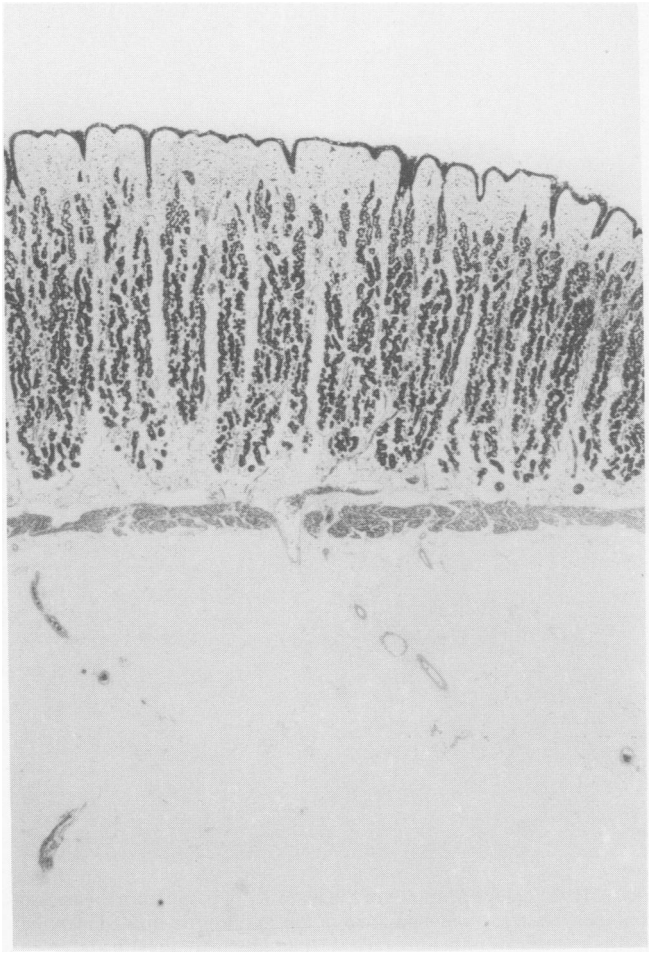

FIG. 1. Tissue from the test chamber illustrating the swollen, edematous appearance of the mucosa following misoprostol administration. The submucosa exhibits a similar reaction. Note the increased height of the mucosa when compared to Figure 2 (methacrylate, MBBF stain, original magnification ×100).

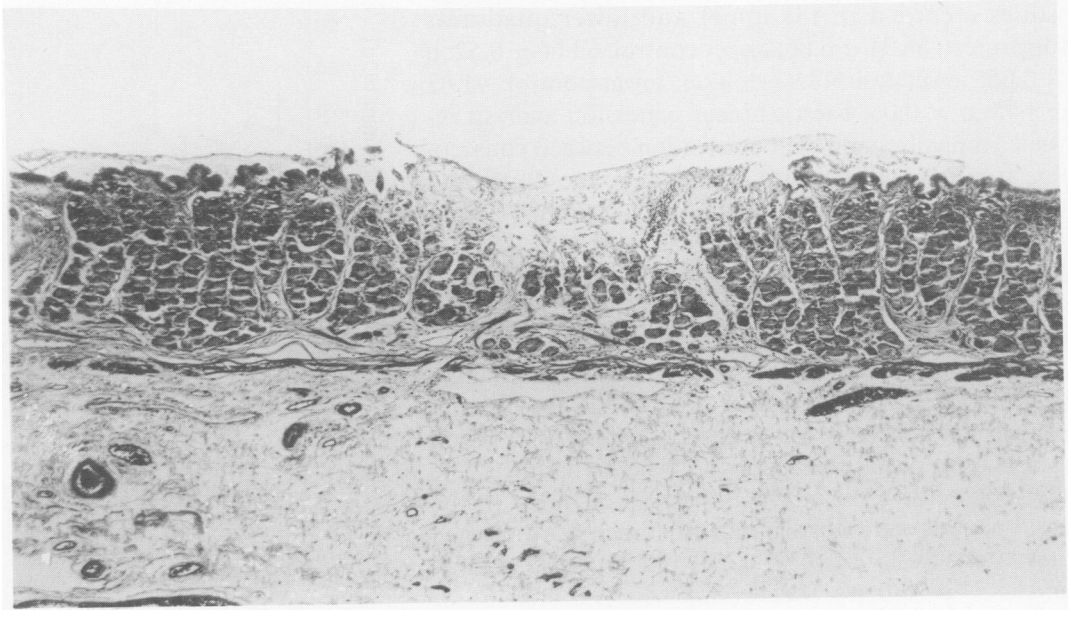

FIG. 2. A section through an aspirin-induced lesion in tissue from the control chamber. Areas adjacent to the lesion as well as the submucosa exhibit otherwise normal morphology (paraffin, hematoxylin and eosin, original magnification ×100).

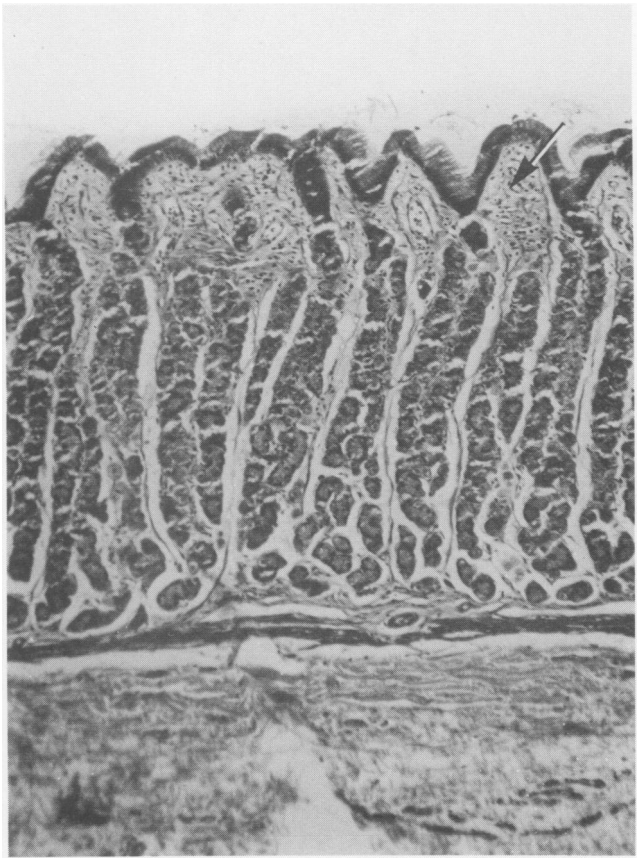

FIG. 3. Histologic appearance of the majority of tissue from the control chamber. Note the widened zone of lamina propria immediately beneath the surface epithelium (arrow) (paraffin, hematoxylin and eosin, original magnification ×160).

mean (± SE) resting GMBF values in the two upper and lower quadrants. Between the two chambers, there were no significant differences in the mean resting GMBF values recorded in the upper and lower quadrants (upper test: 66.31 ± 6.86; upper control: 63.66 ± 6.52; $p > 0.05$; lower test: 48.97 ± 4.08; lower control: 41.67 ± 3.45; $p > 0.05$; t-test). Linear regression analysis revealed a highly significant correlation between consecutive resting GMBF values recorded by the upper and lower electrodes located within each chamber (test: $r = 0.7511$, $N = 13$, $p < 0.01$; control: $r = 0.7151$, $N = 13$, $p < 0.01$), but consecutive resting GMBF values recorded by electrodes located on the same horizontal axis in the two chambers were not correlated.

Figure 4 depicts the simultaneous sequential changes in mean (±SE) GMBF values measured in the test and control chambers during the basal, misoprostol/control, aspirin, and postaspirin periods. In both chambers, the four consecutive mean GMBF values obtained in the basal periods were not significantly different from each other (test: 64.36 ± 10.77, 66.70 ± 9.36, 54.82 ± 7.54, and 55.71 ± 7.80; $p > 0.05$; control: 56.09 ± 6.23, 55.79 ± 7.99, 52.77 ± 9.53, and 48.37 ± 7.58; $p > 0.05$; ANOVA). In the test chamber immediately after the administration of misoprostol, there was a significant and transient increase (18%) in GMBF in the first misoprostol period above the final basal period mean flow value (from 55.71 ± 7.80 to 65.84 ± 6.12; $p < 0.05$; t-test). In the second misoprostol period, the mean GMBF value of 51.27 ± 6.10 was not significantly different from the final basal period mean flow value, indicating a return of GMBF to premisoprostol (basal) levels within 15 minutes even though administration of misoprostol was continued for an additional 15 minutes. After the misoprostol periods focal GMBF in the test chamber showed a graded drop throughout the aspirin periods, became significantly less than the final basal period mean flow value in the second aspirin period, and remained so until the end of the experiments. In the control chamber, there was a graded drop in GMBF throughout the aspirin periods, which became significantly different from the final basal period during the first postaspirin period and remained stable but significantly less than the basal period mean flow value in the remaining periods of the experiments.

Comparison of the simultaneous GMBF values obtained from the two chambers revealed no significant differences except during the first misoprostol/control period (test: 65.84 ± 6.12; control 41.43 ± 6.25; $p < 0.01$; t-test). In the second misoprostol/control period, though the mean GMBF value in the test chamber remained higher than in the control chamber, the difference was not significant statistically. Thereafter with aspirin, GMBF dropped in both chambers and re-

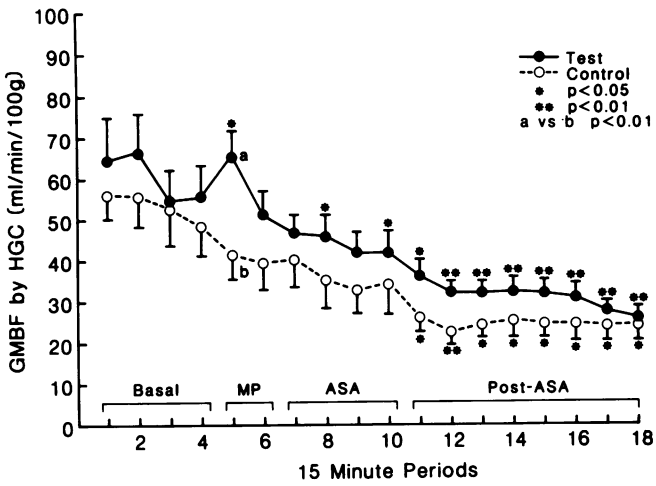

FIG. 4. The simultaneous sequential changes in mean (± SE) GMBF measured in the test and control chambers during the basal, misoprostol/control, aspirin and postaspirin periods. N = 8 for each point. Asterisks indicate significant differences from the final basal period mean (±SE) flow value within each chamber. MP = misoprostol/control periods; ASA = aspirin periods; HGC = hydrogen gas clearance.

TABLE 1. *Net Ionic and Fluid Fluxes in the Test and Control Gastric Chambers*

Periods	Flux Types	Test Chamber	Control Chamber	Test *Versus* Control P Value
Basal	Fluid (mL/15 min)	+0.02 ± 0.15	+0.09 ± 0.18	NS
	Na^+ (μmoL/15 min)	+79.91 ± 25.29	+95.63 ± 29.35	NS
	H^+ (μmoL/15 min)	−64.83 ± 15.69	−69.27 ± 17.38	NS
Misoprostol/control	Fluid (mL/15 min)	+3.36 ± 0.78*	+0.18 ± 0.28	<0.01
	Na^+ (μmoL/15 min)	+593.45 ± 103.21*	+171.42 ± 17.94	<0.01
	H^+ (μmoL/15 min)	−101.27 ± 27.65	−133.51 ± 35.99	NS
Aspirin	Fluid (mL/15 min)	+1.06 ± 0.29†	−0.49 ± 0.22	<0.01
	Na^+ (μmoL/15 min)	+213.52 ± 35.63†	−4.06 ± 11.11†	<0.001
	H^+ (μmoL/15 min)	+5.52 ± 12.02†	−20.53 ± 29.33	NS
Postaspirin	Fluid (mL/15 min)	+0.17 ± 0.07	−0.48 ± 0.11†	<0.001
	Na^+ (μmoL/15 min)	+84.28 ± 10.97	+26.52 ± 8.02†	<0.01
	H^+ (μmoL/15 min)	−45.49 ± 5.63	−88.30 ± 13.90	<0.01

Values are given as mean ± SE. Plus (+) sign denotes a net luminal gain or efflux and a minus (−) sign a net luminal loss or influx.

* Significant difference from the basal period within the same chamber, $p < 0.001$.

† Significant difference from the basal period within the same chamber, $p < 0.01$.

NS = not significant.

mained higher though insignificantly so in the test chamber.

Ionic Fluxes

The results of the net fluxes of ions and fluid are summarized in Table 1. In this text influx denoted by a minus sign in front of the flux value means net luminal loss while efflux denoted by a plus sign means net luminal gain.

Sodium ion flux. In the test chamber immediately after misoprostol administration, there was a highly significant increase in Na^+ efflux into the lumen that was sustained until the end of the aspirin periods. Na^+ efflux returned to basal levels during the postaspirin periods (Fig. 5). In the control chamber during aspirin administration, there was a significant Na^+ influx from the lumen. This was reversed in the postaspirin periods, but the Na^+ efflux remained significantly less than that of the basal period.

Fluid flux across the mucosa behaved in exactly the same way as sodium in the test chamber (Fig. 6). In the control chamber, there was an insignificant fluid influx after the control period.

There were highly significant linear correlations between net changes in Na^+ and fluid in the test ($r = 0.9903$, $N = 18$, $p < 0.001$) and control ($r = 0.8652$, $N = 18$, $p < 0.001$) chambers.

Hydrogen ion flux. In the test chamber there was a significant ($p < 0.01$, t-test) H^+ efflux in the aspirin periods. In the control chamber there was no significant change in the net H^+ flux throughout the experiments. The only difference between the test and control

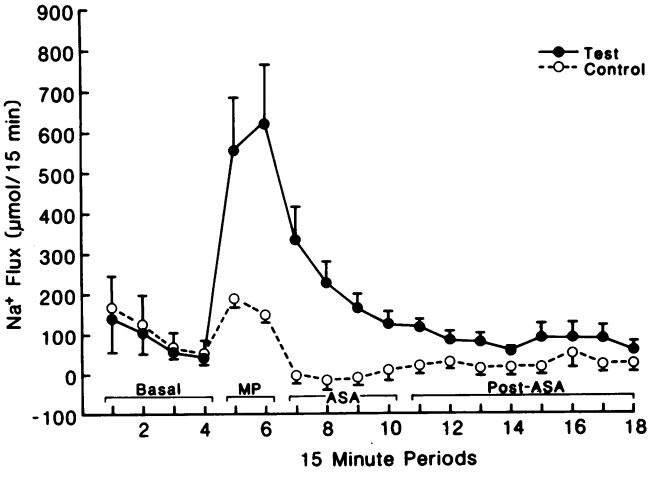

FIG. 5. The sequential changes in the flux of sodium in the test and control chambers during the basal, misoprostol/control, aspirin, and postaspirin periods.

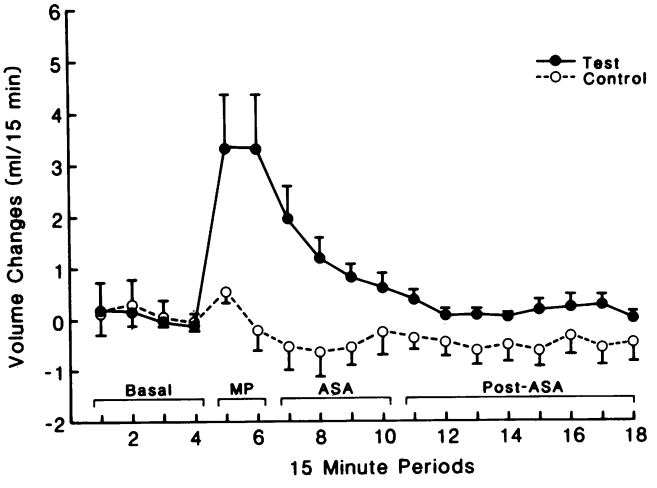

FIG. 6. The sequential changes in the flux of fluid in the test and control chambers during the basal, misoprostol/control, aspirin, and postaspirin periods. The pattern of the changes are identical to that of sodium.

chambers was a significantly higher H^+ influx in the latter in the postaspirin periods.

Relationship Between Gastric Mucosal Blood Flow and Fluxes

In the test chamber no correlations were observed between GMBF and the fluxes of Na^+, H^+, and fluid. In the control chamber, there were highly significant linear correlations between GMBF and fluid flux ($r = 0.7364$, $N = 18$, $p < 0.001$), and GMBF and Na^+ flux ($r = 0.5928$, $N = 18$, $p < 0.01$).

Discussion

Though the precise mechanism(s) of gastric cytoprotection is not known, some experimental evidence has emerged in support of the circulatory hypothesis.[1-6] According to the latter, an increase in GMBF is believed to (1) maintain an adequate supply of oxygen and nutrients to the mucosal cells, thereby preventing a deficit in energy metabolism; (2) supply bicarbonate that will buffer back-diffused hydrogen ions, and (3) rapidly eliminate both back-diffused hydrogen ions and the damaging agent that may have gained access into the subepithelial tissues.[35,36] Discrepancies in the results of previous studies on the role of GMBF in cytoprotection may be due to measurement techniques employed, experimental models, experimental designs, and route of administration of the PG.

HGC is a noninvasive method that has been validated for focal GMBF measurements in animals[37-39] and man.[37] Using this technique in our study after topical administration of misoprostol, we observed an immediate and small transient increase (18%) in focal GMBF. The nature of this increase in blood flow is suggestive of a direct vasodilatory effect by misoprostol. The results of this study differ from that of Leung et al.[27] who also (using HGC) reported no effect by misoprostol on resting GMBF and on GMBF during inhibition of stimulated acid secretion in rats. The differences may be due to the fact that in their study GMBF measurements were commenced 15 minutes after misoprostol treatment. Similar to our results, Sato et al.[31] reported that misoprostol increased gastric mucosal blood volume by 10–25% at various sites in the fundus and antrum as measured by reflectance spectrophotometry in healthy human male volunteers. Likewise, Larsen et al.[2] observed an increase in total gastric blood flow obtained by venous outflow in the canine chambered gastric segment model after topical misoprostol. Colton et al.[23] observed that misoprostol at doses that inhibited histamine-stimulated acid secretion reduced GMBF as measured by aminopyrine clearance, but the ratio (R) of GMBF to rate of acid secretion was actually increased. This indicated that misoprostol has a vasodilating effect in the gastric mucosal vascular bed. Similarly, intra-arterial PGE_1 has been reported to increase significantly total (by venous outflow and radioactive microspheres) and mucosal blood flow (by radioactive microspheres) in canine chambered gastric segment model.[40] Also, topical PGE_1 has been shown to dose-dependently increase superficial gastric mucosal microvascular flow measured by *in vivo* microscopy in rats.[41] The findings in this study confirm these earlier observations that misoprostol, a synthetic PGE_1 analog, is a vasodilator in the gastric mucosal vascular bed.

Our results also confirmed previous reports that misoprostol is cytoprotective.[2,24-26,28-30] The degree of mucosal swelling observed after misoprostol administration was surprising. The histology clearly attributed this gross observation to an edematous reaction within the lamina propria causing increased spacing and separation of the gastric glands. A thick mucoid layer overlying the surface epithelium was also evident in these sections. The aspirin-induced lesions were similar in severity and morphology to those described by other authors.[42] The frequent observation of a widened zone of lamina propria immediately below the surface epithelium is reminiscent of the fashion in which this layer is shed in response to necrotizing agents such as absolute ethanol.[43] The fact that many regions of tissue from the control chambers lacked any evidence of mucosal disruption could be attributed to the random nature of sample site selection as well as the length of the experimental period. It has been shown that epithelial repair under these conditions can begin within 3, and is completed within 60, minutes.[44]

The extent to which GMBF contributes to cytoprotection is unknown. Larsen et al, in the aforementioned study observed an increase in total gastric blood flow by misoprostol of over 400% that was sustained during subsequent aspirin-shock injury, and concluded that the increase in blood flow was responsible for the cytoprotective effects.[2] Recently, we have shown that topical aspirin induces a reduction in GMBF of varying degrees and that mucosal areas with blood flow reduced to below a *critical value* develop gross damage.[34] In this study we were unable to demonstrate that misoprostol pretreatment reverses aspirin-induced decrease in GMBF or maintains blood flow after aspirin administration. Our results therefore do not suggest that this transient vasoactive effect is an important mechanism of gastric cytoprotection.

In terms of ionic fluxes, Colton et al.[24] found no significant difference in the influx of H^+ and Cl^-, and efflux of Na^+ observed with misoprostol and aspirin when administered into canine Heidenhain pouches separately or together. They concluded that influx of H^+ and

efflux of Na^+ from the stomach does not necessarily indicate mucosal damage. On the contrary, Larsen et al.[2] have reported that misoprostol had a negligible effect on the fluxes of Na^+, Cl^-, and H^+ that were not significantly different from the controls. In this study the only consistent flux changes observed were the significant increase in the efflux of Na^+ and fluid into the test chamber lumen immediately after administration of misoprostol. The efflux of Na^+ and fluid could be due to either damage to the gastric mucosal barrier by misoprostol, as was initially attributed to 16,16-dimethyl PGE_2,[45–47] or stimulation of a nonparietal cell secretion rich in Na^+.[48,49] Since no reports have shown that misoprostol damages the mucosa grossly or histologically, and the 200-μg dose of misoprostol used in this study has been experimentally and clinically shown to be cytoprotective, we favor the latter explanation for the observed increase in Na^+ and fluid efflux. The highly significant linear correlation observed in the test chamber between the fluxes of Na^+ and fluid further suggests that these fluxes may be mediated through the same pathway, namely, stimulation of nonparietal secretion rich in Na^+.

We cannot offer any explanation for the findings in H^+ fluxes. It is noteworthy that in the presence of PG, agents that stimulate active secretion of bicarbonate or Na^+-rich fluids,[50,51] disruption of the gastric mucosal barrier by topical damaging agents (*e.g.*, aspirin, alcohol, and bile salts) will not result in the classical picture proposed by Davenport, namely, back diffusion of H^+ and efflux of Na^+ into the lumen.[52] Neutralization of acid by bicarbonate and the large amounts of Na^+ effluxed into the lumen under these circumstances will inevitably alter the net ionic fluxes. This may be the reason for the failure to obtain results consistent with Davenport's hypothesis in this and other studies when an agent that stimulates active secretion of Na^+ bicarbonate is used. This finding also raises questions about the vallidity of using the efflux of Na^+ and the luminal loss of H^+ as indicators of gastric mucosal barrier damage under these circumstances. The linear relationship between GMBF and the fluxes of Na^+ and fluid in the control but not the test chamber may be accounted for by misoprostol since it is the only different variable between the two chambers.

In conclusion, though our results did show a significant and transient increase in GMBF by misoprostol pretreatment, it did not prevent subsequent decrease in GMBF by aspirin. The sustained efflux of Na^+-rich fluids (*i.e.*, nonparietal cell secretion) induced by this synthetic PGE_1 analog may be important in gastric cytoprotection. Our results confirm that misoprostol is vasodilatory and cytoprotective, and it stimulates nonparietal cell secretion. Further studies of the vasoactive effects of PG as an important mechanism of cytoprotection are required.

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