Misoprostol inhibits gastric mucosal release of endogenous prostaglandin E₂ and thromboxane B₂ in healthy volunteers

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Abstract

Prostaglandin analogues of the E-series theoretically offer the ideal antiulcer drugs. Peptic ulcer healing with prostaglandin analogues is, however, no better than would be predicted from their ability to inhibit gastric acid secretion and they are less effective than histamine H₂ receptor antagonists in preventing ulcer relapse. It could be that prostaglandin analogues inhibit gastric mucosal synthesis or release of endogenous eicosanoids, thereby abrogating their own effects. This study, therefore, examined how a single therapeutic dose (200 μg) of misoprostol, a synthetic analogue of prostaglandin E₁, influences gastric mucosal release of endogenous prostaglandin E₂ (PGE₂), thromboxane B₂ (TXB₂), and chemotactic leukotriene B₄ (LTB₄) during basal conditions and in response to gastric luminal acidification (0.1 M HCl; 5 ml/min for 10 minutes). Nine healthy volunteers were studied in a single blind, cross over design. In each subject misoprostol or placebo was instilled in randomised order into the stomach, which was subsequently perfused with isotonic mannitol. Misoprostol significantly decreased basal as well as acid stimulated output of PGE₂ and TXB₂, without affecting output of LTB₄. These data show that misoprostol inhibits gastric mucosal synthesis of prostanooids. Decreased concentrations, or even a changed profile, of native eicosanoids modulating the release of inflammatory mediators from immune cells might explain why prostaglandin analogues have a comparatively poor clinical performance in ulcer healing and prevention.

Methods

Subjects

Ten healthy volunteers (six males and four females, median age 27 years, range 21–46) with no history of peptic ulcer disease consented to the protocol. The study was carried out according to the Helsinki II Declaration and approved by the ethics committee of Copenhagen and Frederiksberg.

Experimental design

All 10 healthy volunteers were studied twice with an interval of at least one week. Because
misoprostol is a potent inhibitor of gastric acid secretion the identity of active and placebo experiments could not remain blinded to the investigator. Consequently, a randomised, single blind, cross over design was used. A 200 µg misoprostol tablet or a placebo tablet of similar appearance (kindly provided by Searle Pharmaceuticals, Morpeth, UK) was dissolved in 10 ml of water and instilled into the stomach one hour before gastric perfusion. Each gastric perfusion included a 30 minute equilibration period followed by a 30 minute basal period and a 45 minute period after acidification of the stomach.

EXPERIMENTAL PROCEDURE

Experiments were performed using a double lumen gastric tube (16 French gauge, AN 10 Anderson Samplers, Atlanta, GA, USA) as described previously in detail.20 After an overnight fast the stomach was intubated. Under fluoroscopic guidance the tip of the tube was placed in the distal antrum. After instillation of misoprostol or placebo the subject rested in a semirecumbent position for one hour before start of perfusion. The stomach was perfused at a rate of 5 ml/min (LKB 2115 Multiperpex Pump, Bromma, Sweden) with an isotonic solution containing mannitol 54 g/l (0-3 M) and [51Cr]-EDTA (10 µCi/ml) as a non- absorbable marker. Aspiration was by intermittent suction from the distal port. During the perfusion saliva was continuously removed by dental suction. After the 30 minute equilibration period 15 minute samples were collected consecutively. For acidification of the stomach 100 mM HCl plus 54 mM NaCl were infused for 10 minutes at a rate of 5 ml/min.

ANALYTICAL PROCEDURES

The volume of the 15 minute effluents was read to the nearest millilitre. The acidity was determined by titration to pH=7.0 with 0-1 M NaOH using an autotitratior (PHM82, Radiometer, Copenhagen, Denmark). [51Cr] activity was measured by gammaspectrometry (Model 1185, Searle Nuclear Chicago Division, Chicago, IL, USA). Samples for determination of PGE₂, TXB₂, and LTβ₄ were neutralised immediately and frozen to −20°C with samples for measurements of trypsin until analysis.51

PGE₂ was measured by a radioimmunological method validated by gas chromatography mass spectrometry as previously described in detail.22 23 Briefly, the method included addition of ³H-labelled PGE₂ (Amersham International, Buckinghamshire, UK) as an internal standard, acidification, extraction with dichloromethane:cyclohexane (2:3), and isolation of LTβ₄ by chromatography on microcolumns of Sephadex LH-20 before performing the radioimmunoassay on the eluate fraction.24

LTβ₄ was determined by the method previously described,24 using another commercially available antibody (Advanced Magnetics, Cambridge, MA, USA). Briefly, the procedure included addition of ³H-labelled LTβ₄ (Amersham International) as an internal standard, acidification, extraction with dichloromethane:cyclohexane (2:3), and isolation of LTβ₄ by chromatography on microcolumns of Sephadex LH-20 before performing the radioimmunoassay on the eluate fraction.

Trypsin was measured in the gastric effluents to assess duodenogastric reflux. A microscale modification of the procedure described by Johnson et al was used.25 This method was based on the rate at which Nω-benzoyl-L-arginine-p-nitroanilide hydrochloride (Merck, Darmstadt, Germany) was hydrolysed by a standard solution of porcine trypsin (Sigma, St Louis, MO, USA). The reaction was performed in microtitration plates (Nunc, Roskilde, Denmark) and quantified spectrophotometrically (Bio-Kinetics reader EL 312e, Bio-Tek Instruments, Winooski, VT, USA) at 405 nm against a substrate/water blank.

CALCULATIONS AND STATISTICAL ANALYSES

Basal fluid, acid, PGE₂, TXB₂, and LTβ₄ outputs were calculated as the means of the results obtained during the two basal periods. Similarly, the results obtained during the periods after luminal acidification were calculated as the means of the first two values. Results are expressed as medians (interquartile ranges).

All data were analysed using analysis of variance techniques (ANOVA) for a two way model with misoprostol and acid stimulation as main effects. The normality assumption for this model was tested with the Kolmogorov-Smirnov statistics. The Levene’s statistic was used to test for equal variance. For some end points, the normality assumption was violated. Thus, the Wilcoxon signed rank test was performed to corroborate the parametric analysis test. p Values from the Wilcoxon test were reported. Values of p<0.05 (two sided test) were considered significant.

Results

RECOVERY AND DUODENOGASTRIC REFLUX
A single subject was excluded from the study because of a low recovery of gastric fluid
The results are expressed as medians (interquartile ranges); n=9.

(<70%) and high trypsin concentrations (that is, more than 22.5 U/15 min equal to 3% of the average normal minimum trypsin output into the duodenum26). In the other nine subjects the results of recovery and trypsin determinations showed no statistically significant differences between placebo and misoprostol experiments or between basal periods and periods after luminal acidification (Table I). The median recovery of gastric fluid output was 88 (78–97)% and the median value for appearance of trypsin into the stomach was 1:1 (0:7–2:1) U/15 min, which equals 0:4% of the average normal minimum trypsin output into the duodenum.

**FLUID AND ACID OUTPUT**

Gastric luminal acidification increased (p<0:01) fluid output compared with basal conditions (Table II). Pretreatment with misoprostol reduced (p<0:02) basal gastric fluid output, while the acid stimulated fluid output was unchanged (p>0:05) compared with the corresponding placebo experiments.

Misoprostol also significantly inhibited (p<0:02) basal gastric acid output (Table II).

**LUMINAL OUTPUT OF PGE2**

In placebo experiments basal luminal output of PGE2 was 7:4 (5:5–10:3) ng/15 min. Luminal acidification increased (p<0:01) basal output of PGE2 to 34:4 (18:3–45:3) ng/15 min.

Misoprostol caused no significant change (p>0:05) in basal luminal concentration of PGE2 but decreased (p<0:02) basal output of PGE2 to 5:6 (3:7–6:6) ng/15 min. After luminal acidification, misoprostol decreased (p<0:02) luminal concentrations of PGE2, which resulted in a considerable reduction (p<0:02) in PGE2 output (14:8 (11:0–16:9) ng/15 min) compared with that seen in placebo experiments (Figure).

**LUMINAL OUTPUT OF TXB2**

In placebo experiments basal luminal output of TXB2 was 9:4 (4:7–23:0) ng/15 min. After luminal acidification the output of TXB2 was 17:3 (13:5–38:5) ng/15 min, which was insignificantly different (p>0:05) from the basal value.

Misoprostol caused no change (p>0:05) in basal luminal concentration of TXB2, but decreased (p<0:05) the basal output of TXB2 to 4:7 (2:3–6:4) ng/15 min. After luminal acidification, misoprostol decreased (p<0:02) luminal concentrations of TXB2, which resulted in a reduction (p<0:02) in TXB2 output (12:8 (8:0–14:9) ng/15 min) compared with that seen in placebo experiments (Figure).

**DISCUSSION**

Since the introduction of the concept of gastric mucosal protection in the early 1970s, considerable effort has been invested in exploring the mechanisms through which prostaglandins may increase the resistance of the gastrointestinal mucosa.27 While there can be little doubt that prostaglandins play a critical physiological part in maintaining the gastrointestinal mucosal integrity,1,8,11 there is no unifying concept to explain which of the numerous proposed mechanisms (for example, secretion of mucus and bicarbonate, epithelial cell proliferation and differentiation, integrity of the microcirculation) is of prime importance and any connection between cytoprotection in animal models and ulcer healing or prevention in humans remains an act of faith.3

The conflicting reports on mucosal prostaglandin formation in peptic ulcer patients probably reflect some of the difficulties inherent in the choice of an appropriate experimental design.1,8,28–32 In this study luminal output of PGE2, TXB2, and LTB4, rather than the content or synthetic capacity of eicosanoids in mucosal biopsies, was measured. This was done (on the assumption that concentrations of eicosanoids in the gastric juice are reflective of mucosal concentrations) because the approach is less traumatizing and minimises non-specific eicosanoid biosynthesis.33 Thus, luminal PGE2 seems to reflect the in vivo balance between mucosal synthesis and degradation, although luminal PGE2 has probably no specific function of its own.

This study was not designed to explore the possibility that mucosal prostaglandin formation may be abnormal in peptic ulcer disease. Nevertheless, the results are highly relevant for the evaluation of the rationale for using prostaglandin analogues in the treatment of peptic ulcer disease and for prevention of mucosal damage associated with the use of NSAIDs. The question whether
administration of prostaglandin analogues may influence gastric prostaglandin synthesis has previously been considered in a single study on the synthesis of PGE₂ and 6-keto-PGF₁α by cultured gastric mucosa obtained from patients with duodenal ulcer. In this study ranitidine increased the in vitro formation of the named prostanooids, while misoprostol caused no change. The results of our study show that misoprostol causes a significant reduction in both basal as well as acid stimulated gastric luminal output of the prostanoids PGE₂ and TXB₂. It might be argued, however, that the reduction in prostanoid output in the presence of acid is a consequence of mucosal protection by misoprostol, rather than interference of misoprostol with gastric mucosal synthesis or release of prostanoids. This explanation seems to be ruled out by the finding of reduced prostanoid output also under basal conditions in misoprostol experiments. The view is supported by other studies in healthy subjects showing that the rise in gastric PGE₂ occurring after a physiological acid load of 0·15 mM results in unchanged DNA output. Conversely, the values of gastric TXB₂ output were not consistently changed by the administration of misoprostol, but it cannot be excluded that a type 2 error, resulting from a considerable variability of low TXB₂ concentrations at the borderline of detection, may have concealed a true difference.

As misoprostol was given topically in excess of native prostaglandins it is difficult to discover if the reduction in concentrations of endogenous prostanoids at the target cells in the gastric mucosa may explain the lesser efficacy of prostaglandin analogues compared with low dose antacid treatment¹⁹ or equipotent antiseretory doses of histamine H₂ receptor antagonists¹⁻³⁻⁵⁻⁷ in healing of peptic ulcers. However, misoprostol significantly reduces the incidence of gastric and duodenal ulcers associated with the use of NSAIDs.¹⁰ As there are considerable differences in the potency of prostaglandin analogues and native prostaglandins as inhibitors of mediators of inflammation¹⁹ changes in concentrations of prostaglandins in the gastric mucosa may disturb complex interactions and balances in inflammatory mediator metabolic pathways. This could be important in the light of the findings suggesting a role for various inflammatory mediators in the pathogenesis of ulcer disease as supported by experimental models and clinical studies.¹¹⁻¹⁹ Theoretically, the trophic actions of prostaglandin analogues, resulting in increased parietal cell mass in rats,³⁶ and the rise in pepsin release caused by prostaglandin analogues,³⁷ may add to the therapeutic problems of this treatment regimen.

So far there has been no other studies on the effects of prostaglandin analogues on gastro-duodenal biosynthesis of thromboxanes or leukotrienes, but a role for thromboxanes in the pathogenesis of ulcer disease has been suggested.⁹ Studies with drugs inhibiting gastric TXA₂ synthesis show that mucosal protection may be a general property of these inhibitors.⁸ In this study misoprostol was shown to inhibit gastric TXB₂ release. On theoretical grounds this effect may be considered beneficial. Decreased concentrations of TXB₂, but not PGE₁ or 6-keto-PGF₁α, have also been found in synovial fluids from patients with effusions of the knee joint treated with a combination of diclofenac and misoprostol, but not if treated with diclofenac alone.³⁸ These data were statistically significant, however, only when analysed within the group. Whether specific thromboxane synthesis inhibitors or thromboxane receptor

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Box plots showing the effect of misoprostol on gastric luminal output of PGE₂ (A), TXB₂ (B), and LTB₄ (C) compared with placebo during basal conditions (open box) and after luminal acidification (0·1 mM HCl, 5 ml/min, 10 minutes) (dotted box) in nine healthy volunteers. The same subjects were studied on separate days. The central line is the group median, the box encloses the middle 50% of the data values, and the tails the most extreme values within a border or 'fence' at 1·5 times the interquartile range. Circles illustrate data outside 10th and 90th percentiles. *p<0·05; †p<0·02.
antagonists may be effective in healing peptic ulcers or preventing gastroduodenal mucosal damage associated with the use of NSAIDs remains to be determined.

In summary, this study shows that the gastric output of PGE₂ and TXB₂ was significantly depressed by the prostaglandin analogue, misoprostol, while the luminal output of LTB₄ was unchanged. Although our study does not identify the mechanism by which misoprostol inhibits endogenous prostanooids a tentative explanation would be that misoprostol antagonises the TXA₂/PG endoperoxide receptor. At any rate, decreased concentrations, or even a changed profile, of native eicosanoids at specific receptors of immune cells in the lamina propria might explain why prostaglandin analogues have a comparatively poor clinical performance in ulcer healing and prevention.

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