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

A Mertz-Nielsen, O Eskerod, K Bukhave, J Rask-Madsen

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 prostanoids. 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.

(Gut 1995; 36: 511-515)

Keywords: misoprostol, prostaglandins, thromboxanes, leukotrienes.

they seemed equal to or even less effective than histamine H₂ receptor antagonists in preventing ulcer relapse.⁴⁻⁶ These findings would imply that the so called cytoprotective properties of prostaglandins, shown in animal experiments⁸ and clinically by a significant reduction in the incidence of the gastric or duodenal ulcers associated with the use of non-steroidal anti-inflammatory drugs (NSAIDs),^{9,10} play no major part in ulcer healing and prevention.

Despite enormous interest in the cytoprotective phenomenon, the mechanism by which prostaglandins produce this effect is still unclear. On the other hand, a role for various inflammatory mediators in the pathogenesis of ulcer disease has been supported by both experimental models and clinical studies.¹¹ For example, platelet activating factor,^{12,13} histamine,^{14,15} and tumour necrosis factor alpha,¹⁶⁻¹⁸ in addition to leukotrienes,¹³ have been implicated as mediators of gastrointestinal injury. Potent inhibitory effects of E-type prostaglandins and the prostaglandin analogue, misoprostol, on the release of pro-inflammatory mediators from, for example, mast cells have also been shown.¹⁹ On theoretical grounds such effects might be beneficial in ulcer treatment and for prevention of NSAID induced gastroduodenal damage, but the administration of a synthetic prostaglandin analogue might also inhibit the synthesis or the release of endogenous eicosanoids, or both, thereby abrogating its own effects. We have, therefore, examined the acute effects of misoprostol, a synthetic analogue of prostaglandin E₁, on gastric release of prostaglandin E₂ (PGE₂), thromboxane B₂ (TXB₂), and leukotriene B₄ (LTB₄), in addition to fluid and acid output, during 'steady state' gastric perfusions of the unstimulated and acid stimulated stomach in healthy subjects.

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

Departments of Medical Gastroenterology, Hvidovre and Herlev Hospitals, University of Copenhagen and Technical University of Denmark, Denmark
A Mertz-Nielsen
O Eskerod
K Bukhave
J Rask-Madsen

Correspondence to:
Dr J Rask-Madsen,
Department of Medical
Gastroenterology 261,
Hvidovre Hospital,
Kettegård Allé 30, DK-2650
Hvidovre, Denmark.

Accepted for publication
15 July 1994

Synthetic prostaglandin analogues of the E-series, in doses recommended for ulcer healing, combine acid inhibitory effects and cytoprotective properties with an ability to inhibit meal stimulated gastrin release.¹⁻⁷ This triade of effects was originally considered to offer the ideal antiulcer drug. Surprisingly, prostaglandin analogues were shown to heal peptic ulcers no better than would be predicted from their ability to inhibit gastric acid secretion^{2,3} and in equipotent antisecretory doses

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 Multiperex Pump, Bromma, Sweden) with an isotonic solution containing mannitol 54 g/l (0.3 M) and [⁵¹Cr]-EDTA (10 µCi/l) 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 autotitrator (PHM82, Radiometer, Copenhagen, Denmark). [⁵¹Cr] activity was measured by gamma spectrometry (Model 1185, Searle Nuclear Chicago Division, Chicago, IL, USA). Samples for determination of PGE₂, TXB₂, and LTB₄ were neutralised immediately and frozen to -20°C with samples for measurements of trypsin until analysis.²¹

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 ethylacetate:cyclohexane (1:1), and chromatography on microcolumns of Sephadex LH-20 (Kabi-Pharmacia AB, Uppsala, Sweden) before performing the radioimmunoassay on the eluate fraction.²² Before the analyses of PGE₂ control experiments were performed with pure misoprostol, which caused no significant cross reaction (that is, <0.01%) after the purification by extraction and chromatography

on microcolumns. Furthermore, addition of pure misoprostol in the range of 0.02–20 ng per test tube during the final radioimmunoassay caused no other interference with the measurement of PGE₂ than a cross reaction of about 0.1%.

TXB₂ was measured by radioimmunoassay as previously described in detail.²⁴ The procedure included addition of ³H-labelled TXB₂ (Amersham International) as an internal standard, acidification, extraction with dichloromethane, and chromatography on microcolumns of Sephadex LH-20 before performing the radioimmunoassay on the eluate fraction.²⁴

LTB₄ 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 LTB₄ (Amersham International) as an internal standard, acidification, extraction with dichloromethane:cyclohexane (2:3), and isolation of LTB₄ by chromatography on microcolumns of Sephadex LH-20 before the radioimmunoassay was performed 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 N^ε-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 LTB₄ 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

TABLE I Recovery of gastric fluid output and appearance of trypsin in the perfused stomach during basal conditions and after luminal acidification (0.1 M HCl, 5 ml/min for 10 minutes)

Period	Placebo		Misoprostol	
	Basal	Acid load	Basal	Acid load
Recovery (%)	90 (77–100)	86 (75–93)	90 (83–98)	84 (78–95)
Trypsin (U/15 min)	1.1 (0.7–1.1)	0.9 (0.7–1.4)	1.4 (0.7–4.5)	1.1 (1.1–1.3)

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).

LUMINAL OUTPUT OF LTB₄

A considerable variability in low concentrations of LTB₄ at the borderline of detection was seen. Neither luminal acidification nor misoprostol changed ($p>0.05$) luminal concentrations or output of LTB₄ significantly (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.⁵

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 traumatising 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

TABLE II Effects of misoprostol on gastric fluid and acid output in the perfused stomach during basal conditions and after luminal acidification (0.1 M HCl, 5 ml/min for 10 minutes)

Period	Placebo		Misoprostol	
	Basal	Acid load	Basal	Acid load
Fluid output (ml/15 min)	14 (11–31)	33 (25–49)†	10 (5–13)*	34 (27–43)†
Acid output (mmol/15 min)	0.7 (0.4–1.4)		0.0 (0.0–0.2)†	

The results are expressed as medians (interquartile ranges); n=9. * $p<0.02$ compared with placebo; † $p<0.001$ compared with basal.

Box plot showing the effect of misoprostol on gastric luminal output of PGE_2 (A), TXB_2 (B), and LTB_4 (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. * $p < 0.05$; † $p < 0.02$.

administration of prostaglandin analogues may influence gastric prostaglandin synthesis has previously been considered in a single study on the synthesis of PGE_2 and 6-keto-PGF $_{1\alpha}$ by cultured gastric mucosa obtained from patients with duodenal ulcer.²⁹ In this study ranitidine increased the *in vitro* formation of the named prostanoids, 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 PGE_2 and TXB_2 . 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 PGE_2 occurring after a physiological acid load of 0.15 mM results in unchanged DNA output.³⁴ Conversely, the values of gastric LTB_4 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 LTB_4 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 treatment³⁵ or equipotent antisecretory doses of histamine H_2 receptor antagonists^{1-3 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.⁸ Studies with drugs inhibiting gastric TXA_2 synthesis show that mucosal protection may be a general property of these inhibitors.⁸ In this study misoprostol was shown to inhibit gastric TXB_2 release. On theoretical grounds this effect may be considered beneficial. Decreased concentrations of TXB_2 , but not PGE_1 or 6-keto-PGF $_{1\alpha}$, 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

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 PGE_2 and TXB_2 was significantly depressed by the prostaglandin analogue, misoprostol, while the luminal output of LTB_4 was unchanged. Although our study does not identify the mechanism by which misoprostol inhibits endogenous prostanoids a tentative explanation would be that misoprostol antagonises the TXA/PG endoperoxide receptor.³⁹ 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.

Part of this work was presented at the Annual Meeting of the American Gastroenterological Association in San Francisco in May 1992 (*Gastroenterology* 1992; 102: A125) and was supported by The Danish Medical Research Council (grant no 12-0219).

- Rask-Madsen J, Lauritsen K. Enhancement of mucosal defense by prostaglandins. Rationale and clinical experience in ulcer disease. *Scand J Gastroenterol* 1987; 22 (suppl 28): 34-42.
- Lauritsen K, Laursen LS, Havelund T, Bytzer P, Svendsen LB, Rask-Madsen J. Enprostil and ranitidine in duodenal ulcer healing: double-blind comparative trial. *BMJ* 1986; 292: 864-6.
- Walt RP, Pounder RE, Hawkey CJ, Sontana IA, Somerville KW, Logan RF, et al. Twenty-four-hour intragastric acidity and clinical trial of bedtime enprostil 70 µg compared with ranitidine 300 mg in duodenal ulcer. *Aliment Pharmacol Ther* 1987; 1: 161-6.
- Lauritsen K, Havelund T, Laursen LS, Bytzer P, Kjærgaard J, Rask-Madsen J. Enprostil and ranitidine in prevention of duodenal ulcer relapse: one year double blind comparative trial. *BMJ* 1987; 294: 932-4.
- Hawkey CJ, Walt RP. Prostaglandins for peptic ulcer: a promise unfulfilled. *Lancet* 1986; ii: 1084-7.
- Lauritsen K, Rask-Madsen J. Prostaglandin analogues. *Baillieres Clin Gastroenterol* 1988; 2: 621-8.
- Walt RP. Misoprostol for the treatment of peptic ulcer and antiinflammatory-drug-induced gastroduodenal ulceration. *N Engl J Med* 1992; 327: 1575-80.
- Hawkey CJ, Rampton DS. Prostaglandins and the gastrointestinal mucosa: are they important in its function, disease, or treatment? *Gastroenterology* 1985; 89: 1162-88.
- Graham DY, Agrawal NM, Roth SH. Prevention of NSAID-induced gastric ulcer with misoprostol: multicentre, double-blind, placebo-controlled trial. *Lancet* 1988; ii: 1277-80.
- Graham DY, White RH, Moreland LW, Schubert T, Katz R, Jaszewski R, et al. Duodenal and gastric ulcer prevention with misoprostol in arthritis patients taking NSAIDs. *Ann Intern Med* 1993; 119: 257-62.
- Wallace JL. Lipid mediators of inflammation in gastric ulcer. *Am J Physiol* 1990; 258: G1-11.
- Wallace JL, Steel G, Whittle BJR, Lagente V, Vargaftig B. Evidence for platelet-activating factor as a mediator of endotoxin-induced gastrointestinal damage in the rat. Effects of three platelet-activating factor antagonists. *Gastroenterology* 1987; 93: 765-73.
- Konturek SJ, Brzozowski T, Drozdowicz D, Garlicki J, Beck G. Role of leukotrienes and platelet activating factor in acute gastric mucosal lesions in rats. *Eur J Pharmacol* 1989; 164: 285-92.
- Franco-Brown S, Masson GMC, Corcoran AC. Induction of acute gastric lesions by histamine liberators in rats: effects thereon of pharmacologic blocking agents. *J Allergy* 1959; 30: 1-10.
- Bommelaer G, Guth PH. Protection by histamine receptor antagonists and prostaglandin against gastric mucosal barrier disruption in the rat. *Gastroenterology* 1979; 77: 303-8.
- Tracey KJ, Beutler B, Lowry SF, Merryweather J, Wolpe S, Milsark IW, et al. Shock and tissue injury induced by recombinant human cachectin. *Science* 1986; 234: 470-4.
- Sun X, Hsueh W. Bowel necrosis induced by tumor necrosis factor in rats is mediated by platelet-activating factor. *J Clin Invest* 1988; 81: 1328-31.
- Mahatma M, Agrawal N, Dajani EZ, Nelson S, Nakamura C, Sitton J. Misoprostol but not antacid prevents endotoxin-induced gastric mucosal injury: role of tumor necrosis factor- α . *Dig Dis Sci* 1991; 36: 1562-8.
- Hogaboam CM, Bissonnette EY, Chin BC, Befus AD, Wallace JL. Prostaglandins inhibit inflammatory mediator release from rat mast cells. *Gastroenterology* 1993; 104: 122-9.
- Mertz-Nielsen A, Steenberg P, Neumark T, Bukhave K, Rask-Madsen J. Colloidal bismuth subcitrate causes sustained release of gastric mucosal prostaglandin E_2 . *Aliment Pharmacol Ther* 1991; 5: 127-33.
- Rask-Madsen J, Bukhave K, Madsen PER, Bekker C. Effect of carbenoxolone on gastric prostaglandin E_2 levels in patients with peptic ulcer disease following vagal and pentagastrin stimulation. *Eur J Clin Invest* 1983; 13: 351-6.
- Bukhave K, Rask-Madsen J. Prostaglandin E_2 in jejunal fluids and its potential diagnostic value for selecting patients with indomethacin-sensitive diarrhoea. *Eur J Clin Invest* 1981; 11: 191-7.
- Bukhave K, Grn K, Rask-Madsen J. Comparison of radioimmunochemical determinations with gas chromatography mass spectrometry dosage. A study of PGE_2 and $PGF_{2\alpha}$ in gastrointestinal fluids. *Biomed Mass Spectrom* 1983; 10: 256-8.
- Lauritsen K, Laursen LS, Bukhave K, Rask-Madsen J. In vivo profiles of eicosanoids in ulcerative colitis, Crohn's colitis, and Clostridium difficile colitis. *Gastroenterology* 1988; 95: 11-7.
- Johnson LA, Deyoe CW, Hoover WJ, Schwenke JR. Inactivation of trypsin inhibitors in aqueous soybean extracts by direct steam infusion. *Cereal Chemistry* 1980; 57: 376-9.
- DiMaggio EP. Alterations and consequences of interdigestive and post-stimulatory human exocrine pancreatic secretion in health and disease. In: Polak JM, Bloom SR, Wright NA, Butler AG, eds. *Basic science in gastroenterology. Physiology of the gut*. Ware, Herts: Glaxo Group Research, 1984: 251-66.
- Robert A. Cytoprotection by prostaglandins. *Gastroenterology* 1979; 77: 761-7.
- Ahlquist DA, Dozois RR, Zinsmeister AR, Malagelada J-R. Duodenal prostaglandin synthesis and acid load in health and in duodenal ulcer disease. *Gastroenterology* 1983; 85: 522-8.
- Rachmilewitz D, Ligumsky M, Fich A, Goldin E, Eliakim A, Karmeli F. Role of endogenous gastric prostanoids in the pathogenesis and therapy of duodenal ulcer. *Gastroenterology* 1986; 90: 963-9.
- Hillier K, Smith CL, Jewell R, Arthur MJP, Ross G. Duodenal mucosa synthesis of prostaglandins in duodenal ulcer disease. *Gut* 1985; 26: 237-40.
- Sharon P, Cohen F, Zifroni A, Karmeli F, Ligumsky M, Rachmilewitz D. Prostanoid synthesis by cultured gastric and duodenal mucosa: possible role in the pathogenesis of duodenal ulcer. *Scand J Gastroenterol* 1983; 18: 1045-9.
- Bukhave K, Rask-Madsen J, Hogan DL, Koss MA, Isenberg JL. Proximal duodenal prostaglandin E_2 release and mucosal bicarbonate secretion are altered in patients with duodenal ulcer. *Gastroenterology* 1990; 99: 951-5.
- Rask-Madsen J, Bukhave K. The difficulties of establishing the pathophysiologic role of prostaglandins in secretion. In: Skadhauge E, Heintze K, eds. *Intestinal absorption and secretion*. Lancaster: MTP Press, 1984: 453-67.
- Aly A, Grn K, Johansson C. Acid instillation increases gastric luminal prostaglandin E_2 output in man. *Acta Med Scand* 1985; 218: 505-10.
- Caspary WF, Hengels KJ, Kunert H, Rsch W, Rohner HG, Spuhler S, et al. Low-dose antacid therapy in the treatment of duodenal ulcer - a multicentre double-blind trial vs misoprostol. *Z Gastroenterol* 1991; 29: 411-6.
- Helander HF, Johansson C, Blom H, Uribe A. Trophic actions of E_2 prostaglandins in the rat gastrointestinal mucosa. A quantitative morphologic study. *Gastroenterology* 1985; 89: 1393-9.
- Basson MD, Zucker KA, Adrian TE, Zdon MJ, Ballantyne GH, Modlin IM. Pepsin release by prostaglandin E_1 analogue. A potential therapeutic problem. *Arch Surg* 1988; 123: 431-3.
- Doube A, Davies J, Notarianni L, Holgate K, Fenn GC. Effect of misoprostol on concentrations of prostaglandins in synovial fluid. *Ann Rheum Dis* 1991; 50: 797-9.
- Vilaseca J, Salas A, Guarner F, Rodrguez R, Malagelada J-R. Participation of thromboxane and other eicosanoid synthesis in the course of experimental inflammatory colitis. *Gastroenterology* 1990; 98: 269-77.