Prostaglandin concentrations in the gastro-duodenal mucosa of asymptomatic human volunteers: the effect of drugs

Ali GOREN, A.P. McKAY, M. SHORTHOUSE, A.J. LEE, J.O. HUNTER

Department of Gastroenterology, Addenbrooke's Hospital, CAMBRIDGE - UK

Prostaglandins (PGs) are believed to have a cytoprotective effect in the stomach and the frequency of peptic ulceration is greater in patients using drugs which inhibit PG synthetase. However, direct studies of PG concentrations in the mucosa of the healthy human stomach are few. Biopsies of the mucosa were taken at gastroscopy from 4 sites (duodenum, antrum, lesser curve, body of stomach) in 46 asymptomatic volunteers age 19 to 33, whose stomachs appeared macroscopically normal. The biopsies were immediately frozen in liquid nitrogen and stored -700 before extraction and determination of the concentration of PGs Ei, *Ez 6-keto* Fia *and* F2o. *and Thromboxane* B2 *by radioimmunoassay. 7 volunteers subsequently received aspirin 900mg t.d.s. and 8 aspirin 900mg t.d.s. plus PGE-\ analogue oxoprostol (May & Baker) 1mg b.d. and 25 indomethacin 25mg t.d.s. and tiaprofenic acid 200mg t.d.s. and 6 cimetidine 400 b.d. and the oxoprostol 1mg b.d. All treatment courses were for 7 days. PG concentrations before treatment expressed as pg/mg protein showed a wide variation between individuals and biopsy sites (*E2 *198-21, 000pg/mg, 6-keto* Fi *71-10,000pg/mg,* F2 *20-50,000 pg/mg, Thromboxane Bz 10-6,000 pg/mg). E1 was detected in very low concentrations in less than 25% The significant changes (p=<0.05, Wilcox's Rank Test) in PG concentrations after drug administration were as follows. Aspirin produced a profound fall in each PG. Tiaprofenic acid reduced* E2 *and 6-keto* F1 a *in duodenum, antrum and body of stomach. Indomethacin reduced 6-keto* F1 a *in duodenum and body only, but the reduction* of £2 *did not reach statistical significance. These results confirm in vivo the supected effects of ulcerogenic drugs on PGs in the normal human gastro-duodenal mucosa. [Turk J Med Res 1993; 11(1): 21-26]*

Key Words: Prostaglandins, Indomethacin, Aspirin, Tiaprofenic acid, Cimetidine, Oxoprostol

Human gastrointestinal mucosa has been shown to contain prostaglandins (PGs) (1.2) which may play a role in the regulation of luminal secretion, especially in the stomach, and protection of the mucosa against physical or chemical injury (3).

Antisecretory PGs can prevent experimentally induced ulcers caused by a variety of acid-dependent and acid-independent agents, such as non-steroidal ant-inflammatory drugs (NSAIDs), bile acids, ethanol, and hyperosmolar solutions (4). This phenomenon is called "cytoprotection".

Aspirin and related NSAIDs are potent inhibitors of PG synthesis (7,8) which effect the cyclooxygenases enzymes to prevent PGs production (9). However, direct in vivo measurements of the concentrations of prostaglandins in gastric and duodenal mucosa in man have been lacking.

This study was designed to determine the distribution of PGs in duodenal and gastric mucosa of healthy subjects, and to examine the effects of certain NSAIDs (aspirin, indomethacin, tiaprofenic acid), cimetidine and (a PGE1 analogue) oxoprostol administration on the generation of mucosal PGs.

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Correspondence: Ali GÖREN
Department of Gastroenterology,
Medical School of Ankara University
Sıhhiye, 06100 ANKARA

MATERIALS AND METHODS

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Prostaglandin Analysis

Biopsies obtained from the antrum were frozen in liquid nitrogen (N2) within 20-30 seconds. The specimens were stored at -70°C until all the specimens to complete the study had been collected. They were then all extracted and analysed in one batch. No demonstrable change in the prostaglandins E2 and 6 keto Fia have been found when specimens were stored for at least 3 months at -70°C.

Extraction: all manipilations were carried out at 0-4C. 2 biopsy specimens from each subject were homogenized by hand in a small glass homogenizer containing 1ml 0.05 M Tris buffer pH 7.2.2ml of ice cold acetone was added. The precipitated proteins were centrifuged down and analysed for protein content. The supernatant was extracted with twice the volume of Petroleum Ether (40-60°C). The Petroleum Ether layer was discarded. The aqueour layer was brought to pH 3.0 with dilute formic acid and extracted with twice the volume of ethyl acetate. The ethyl acetate layer was seperated off and the aqueous layer further extracted with twice the volume of ethyl acetate.

The ethyl acetate layers were combined and taken to dryness under N2. Samples were suspended in radioimmunoassay buffer (0.1 M phosphate buffer containing sodium chloride 0.9%, sodium azide 0.1%, gelatine 0.1%) and submitted to assay using the following: anti sera E_2 with specificity of 100% and cross reactivity with E_1 of 4% anti serum 6 keto F_1 with a specificity of 100% and cross reactivity of 3%, with E_2 (Biosys S.A. France). Each sample was analysed in duplicate and the results expressed as pg of PG per mg protein.

Prostaglandin antisera were suppled by Biopsys S.A. and Sigma France, (1251) labelled PGE_2 antigen from N.E.N. Products USA. Other labelled antigens were suppled by Amersham pic (UK) and unlabelled prostaglandins by Sigma. All other chemicals were from BDH.

Study 1 normal prostaglandin concentration

46 healthy men aged between 19 and 33 with no history of gastrointestinal disease or bleeding disorders were recruited from the staff of the hospital. They were taking no medication and had no known drug intolerances before admission to the study. Each volunteer undewent a full medical history and physical examination, including blood tests, urinalysis and ECG.

Alcohol was forbidden one week before and during the study. Volunteers were fasted from midnight on the evening of the day of endoscopy. Just prior to endoscopy, subjects were given Diazepam 20mg and Buscopan 40mg intravenously.

Endoscopies were performed by a single endoscopist using Olympus Q10 gastroduodenoscope. The oesophagus, fundus and antrum and the duodenum was examined and volunteers showing any abnormality were excluded. Biopsies were taken from the duodenum, antrum, body and lesser curve. Four pieces of tissue approximately 3mm in diameter were obtained from each site.

Study 2 the effects of drugs

The 46 normal volunteers were divided into groups for assessment of the effects of drugs on gastric prostaglandins. In each group, drugs were administered orally in the dosage recorded below for 7 days and a repeat endoscopy perormed, one hour after the administration of the final drug dose. Biopsies were taken from the same sites and in the same way as in study 1. The drug treatment groups were as follows:

Group A: 25 subjects entered a double blind cross over configuration between indomethacin 25mg t.d.s. and tiaprofenic acid 200mg t.d.s. There was a recovery period of one week between the cources of the two drugs.

Group B: Received aspirin 900mg t.d.s. and a placebo for one week or aspirin 900mg t.d.s. and oxoprostol 1mg b.d.

Group C: Received cimetidine 400mg b.d. and oxoprostol 1mg b.d. with a recovery period of one week between the courses of treatment.

All studies were approved by the Ethical Committee of the Cambridge Health Authority and informed written consent was obtained for each participant after the nature of the study had been fully explained.

Statistical Analysis

The results of each experiment were analysed using Wilcoxon's signed rank sum test. Differences were considered significant at $p<0.05$.

RESULTS

The concentrate of PGs in the stomachs of the normal volunteers is shown in Table 1. There was a wide variation between individuals and between biopsy sites. The predominant PG was PGE_2, although prostaglandin was also present in significant quantities (Fig. 1). Prostaglandin F a and thromboxane B_2 were found in much lesser concentrations, and PGE_1 was found in only trace amounts in 25% of our volunteers and has not been considered further.

Oxoprostol and cimetidine produced no significant effect on PG concentrations all the NSAIDs however, dramatically reduced PG concentrations in all areas of the stomach and the duodenum. Oxoprostol did not prevent the effect of aspirin in reducing PG levels (Fig. 2A). No difference was found between the effect on PGs of any individual NSAID, although aspirin alone produced the most dramatic reductions in concentration (Fig. 2B).

DISCUSSION

The gastrointestinal mucosa synthesize and contains substantial amounts of PGs, thromboxanes (Tx) and leukotrienes whose physiological functions are closely interrelated (9,10). Our studies have revealed that gastric and duodenal mucosa producing and containg relatively large amounts of PGs (E_2, 6-keto F_1, F_2 and TxB_2 and negligible amounts of PGE_1), and these

Table 1. Prostaglandin concentration in normal subjects

PGs	Lesser Curve				Body			
	Tr No.	Mean±SE	. No.	Mean-SE	.No	Mean*SE	.No	Mean±SE
E,	46	2471.0 - 346.4	46	4037.0 Ì 742.0	46	2780.0 - 528:2	i 25	5322.6 4 ...'9
S keto	46	1375.6 - 223.7	46	2070.8 - 326.3	46	1302.9 - 267.1	1 25	3120.4 t 594.8
r/xB,	10	266.4 - 110.2	10	527.1 i 233.5	10	252.4 * 137.4	10	378.0 - 194.9
·2	10	491.1 ¢ 370.2	10	511.6 - 320.9	10	117.7 * 38.7	10	426.7 - 246.2
E,	25	584.1 - 331.8	25	1491.5 - 869.2	25	505.3 - 352.8	25	1110.9 - 417.0
6 keto	25	249.3 - 57.4	25	593.5 - 267.0	25	198.2 - 67.4	25	497.8 -132.1
rxB,	10	182.6 - 126.5	10	1103.1 -1009.6	10	263.6 - 248.0	10	42.3 * 17.8
·2	10	83,5 - 49.7	10	80.4 - 42.5	10	47.6 - 24.8	10	108.2 - 50.5
E,	25	544.0 - 278.3	25	467.0 - 218.5	25	630.3 - 320.3	25	663.2 ±310.0
6 keto	25	256.2 i 107.9	25	235.6 - 59.5	25	273.8 - 84.2	25	357 Ì 92.6
TxB,	10	94.3 - 59.9	10	121.0 - 77.2	10	145.3 - 95.7	10	74.7 - 44.8
·2	10	113.3 - 70.4	10	133.9 * 32.8	10	243.0 - 174.2	10	228.3 - 92.2

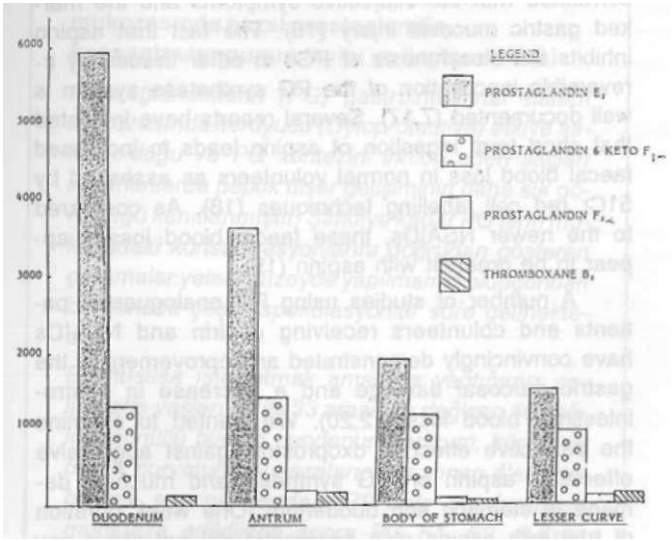

Figure 1. Pre-treatment prostaglandin levels.

were widely different from person to person and area from which they were biopsied.

There is no ideal way of measuring PG content in human tissues. The trauma of taking a biopsy and homogenising the tissue will itself activate the cyclooxygenase pathway leading to the sponaneous generation of PGs. Attempts to get round this problem by measuring PG synthesis by cell cultures or tissue homogenates are often inaccurate if degradation is not simulataneously measured and although measurement of both has been attempted (11) the disadvantage of all in vitro determinations remains that the concentraitons of precursors and cofactors may be very different to those obtaining in vivo.

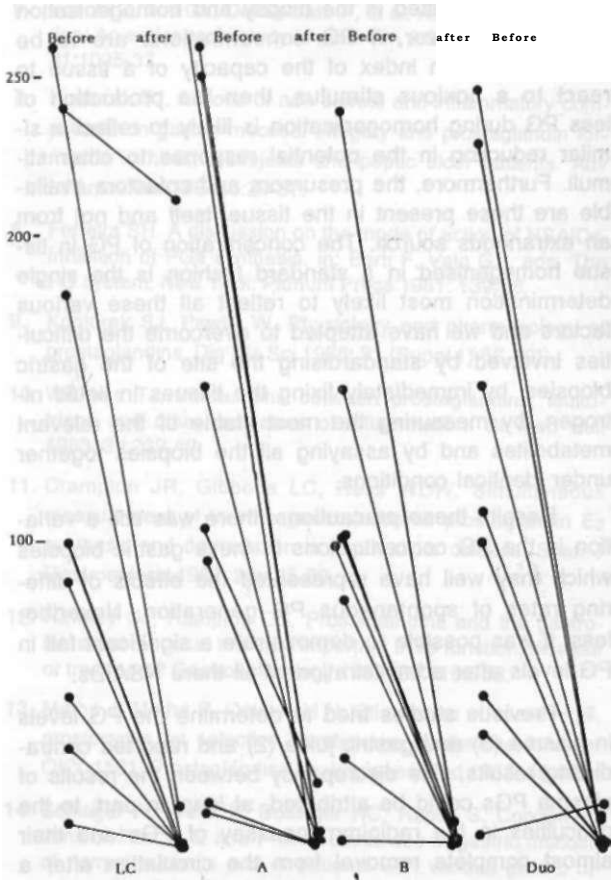

Figure 2A. Levels of PGE2 after aspirin+oxoprostol (n-8 subjects).

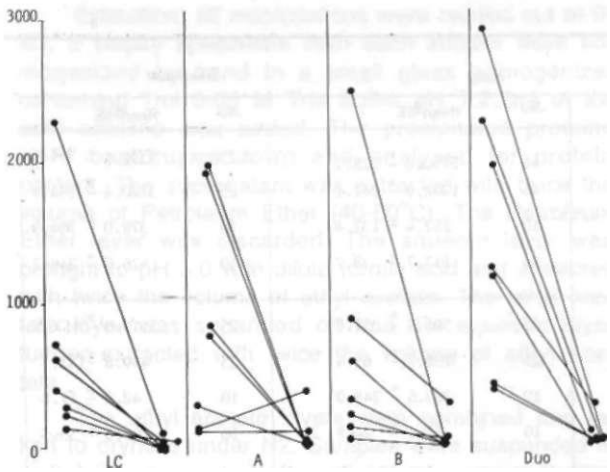

Figure 2B. Levels of 6 keto $F_{i\alpha}$ after aspirin administration (n-7 subjects).

Of the techniques we believe that the direct measurement is likely to be most representative. Hawkey and Rampling (12) have argued that direct tissue measurements of PGs are unreliable in that the PGs measured are not those present in the resting tissue, but those generated in the biopsy and homogenisation process. However, if PG concentrations are to be measured as an index of the capacity of a tissue to react to a noxious stimulus, then the production of less PG during homogenisation is likely to reflect a similar reduction in the potential response to other stimuli. Furthermore, the presursors and cofactors available are those present in the tissue itself and not from an extraneous source. The concentration of PG in tissue homogenised in a standard fashion is the single determination most likely to reflect all these various factors and we have attepted to overcome the difficulties involved by standardising the site of the gastric biopsies, by immediately fixing the tissues in liquid nitrogen, by measuring the most stable of the relevant metabolites and by assaying all the biopsies together under identical conditions.

Despite these precautions, there was still a variation in the PG concentrations in these gastric biopsies which may well have represented the effects of differing rates of spontaneous PG generation. Nevertheless, it was possible to demonstrate a significant fall in PG levels after administration of all there NSAIDs.

Previous studies tried to determine the PG levels in plasma (3) and gastric juice (2) and reported contradicting results. The discrepancy between the results of plasma PGs could be attributed, at least in part, to the difficulties in the radioimmunoassay of PGs and their almost complete removal from the circulation after a single passage through the lungs (13). It has been reported that, the stimulated gastric acid output is significantly correlated with the output of PGE_2 into gastric juice, suggesting that the mucosal PGs may play a role in the local control of gastric acid secretion (9).

We have compared mucosal PGs in gastric and duodenal mucose before and after administration of drugs. If PGs are important in maintaining the integrity of gastrointestinal mucosa, reproducible basal PG levels in the tissue should be determined. Reports concerning PG levels in the gastric and duodenal mucosa of patients with peptic ulcer show variations. Both high (14) and low (7) values have been reported.

The confirmation of the important role of tissue PGs in mucosal integrity comes from the studies on the prevention by exogenous PGs of mucosal damage caused by NSAIDs (15). Several studies on healthy subjects or on arthritis patients showed that oral administration of PGE_2 greatly reduced the bleeding rate and DNA loss as well as decreasing the extent of endoscopic mucosal lesions caused by NSAIDs (16).

The major finding of this study is the demonstration that aspirin causes dramatic and profound reduction in the capacity of the gastric and duodenal mucosa to biosynthesize PGs and that it may be closely correlated with the subjective symptoms and the marked gastric mucosal injury (16). The fact that aspirin inhibits the biosynthesis of PGs in other tissues by irreversible inactivation of the PG synthetase system is well documented (7,17). Several reports have indicated that short term ingestion of aspirin leads to increased faecal blood loss in normal volunteers as assessed by ^{51}Cr red cell labelling techniques (18). As compared to the newer NSAIDs, these faecal blood losses appear to be greatest with aspirin (19).

A number of studies using PG analogues in patients and colunteers receiving aspirin and NSAIDs have convincingly demonstrated an improvement in the gastric mucosal damage and a decrease in gastrointestinal blood loss (12,20). We wanted to examine the protective effect of oxoprostol against aggressive effects of aspirin on PG synthesis and mucosal damage in stomach and duodenum. One week duration of trial with aspirin plus oxoprostol did not show any positive benefit of oxoprostol on the aspirin related damage.

Currently it has been reported that in human subjects cimetidine, in additon to its antisecretory effects, may enhance ulcer healing by promoting and increasing endogenous gastric PG synthesis resulting in increased cytoprotection (21). In part of this study, cimetidine has been given for one week, and gastric mucosal PGs measured before and after trial. However, results did not show any significant change in any PGs measured in thes study. More studies of the effect of cimetidine on PG synthesis and secretion are needed.

NSAIDs other than aspirin, such as indomethacin and other noxious agents were shown to induce damage to gastrointestnal mucosa (22). Most NSAIDs inhibit the cyclooxygenase system, thus causing deple-

tion of tissue PGs. Impairment of those PGs assumed to have cytoprotective propertes may predispose teh mucosa of the gastrointestinal tract to various insult (23), such as erosions and bleeding which have been explained, in part, by the suppression of the generation of PGs (19). Our results with indomethacin indicate that, indeed. NSAIDs were accompanied by a significant reduction, at least some part of gastroduodenal mucosa, in the PG biospynthesis.

Original in vitro studies suggested that tiaprofenic acid selectively inhibits PG synthesis, being 10 times more potent than indomethacin at inhibiting the formation of PGE2 and PGF2 whilst being only half as active as indomethacin in inhibiting the mucosal protective PGE2 (prostacyclin). Our results were not compatible with previous reports. However, we have been able to show that PGE2 and 6-keto Fia were dramatically and profoundly reduced by both indomethacin and tiaprofenic acid.

Sağlıklı gönüllülerde mide duodenum mukozasında bazal prostaglandin konsantrasyonunun tayini ve ilaçların etkisi

Prostaglandinlerin (PG) gastrointestinal sistem mukozasında koruyucu (Cytoprotective) etkiye sahip olduğu ve PG sentezini inhibe eden ilaçları kullananlarda peptik ülser gelişiminin daha sık görüldüğü kanıtlanmıştır. Şimdiye kadar PG 'lerin mukozadaki konsantrasyonlarını doğrudan gösteren çalışmalar yeterli düzeyde yapılmamış olduğundan bu konuda çeşitli spekülasyonlar süre gelmektedir.

Bu hususa ışık tutmak amacıyla yaptığımız çalışmada yaşları 19 ile 33 arasında değişen 46 sağlıklı gönüllü bireyin duodenum, antrum, küçük ve büyük curvatura mukozalarından alınan 4'er adet biyopsi sıvı nitrojende —70°C'de dondurularak muhafaza edildikten sonra PG E1, E2, 6-Keto F1o, F2a ve tromboxan B2'nin bazal konsantrasyonları radioimmunoassey yöntemiyle tayin edildi. Daha sonra bu bunlardan 7'sine 3x900 mg aspirin, 8'inde 3x25 mg indomethacin, 10'unda 3x200 mg tiaprofenic asit, 6'sına 2x400 mg simetidin ve 6'sına oxoprostol (PGE\ analoğu) 2x1 mg verildi. Her bir programa 7 gün süre ile devam edildi. Tedavi öncesinde ve sonrasında PG düzeyleri pikogram/mg-protein olarak ifade edildi. Neticeler kişiden kişiye ve biyopsi bölgelerine göre çok büyük farklılık ve geniş dağılım gösterdi: Aspirin her bir PG seviyelerinde çok belirgin düşmeye sebep oldu. Tiaprofenic asit PGE2 ve 6-Keto Fla'yı duodenum ve büyük curvaturada istatistiki yönden anlamlı ölçüde düşürdü. Simetidin ve Oxoprostolün belirgin etkisi gözlenmedi.

Bu neticeler PG'leri inhibe eden ülserojenik ilaçların canlı dokuda (invivo) etkileşimlerini göstermesi PG'lerin seviyesini düşürerek cytoprotection'u bozması açısından önemli bulunmuştur.
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REFERENCES

1. Bennett A, Stamford IF, Unger WG. Prostaglandin E2 and gastric acid secretion in man. J Physiol 1973; 229:349-60.
2. Cheung LY, Jubiz W, Moore JG. Gastric prostaglandin E2 output during basal and stimulated acid secretion in normal subjects and patients with duodenal ulcer. J Surg Res 1976; 20:369-72.
3. Baker R, Jaffe BM, Venables CW. Endogenous prostoglandins in peptic ulcer disease. Gut 1979; 20:294-9.
4. Robert A, Nezamis JE, Lancaster C, Hanchar AJ. Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl and thermal injury. Gastroenterology 1979; 77:433-43.
5. Robert A. Cytoprotection by prostaglandins. Gastroenterology 1979; 77:661-7.
6. Foschi D, Toti GL, Del Soldato P, et al. Adaptive cytoprotection: An endoscopic study in man. Am J Gastroenterol 1986; 81:1035-37.
7. Konturek SJ. Actions of non steroid anti-inflammatory compounds on gastric mucosal integrity and prostaglandin formation in healthy subjects and peptic ulcer patients. Adv Inflamm Res 1984; 6:29-37.
8. Ferreira SH. A discussion on the mode of action of NSAIDs. Inhibition of PGs synthesis. In: Berti F, Velo GP, eds. The PG system. New York: Plenum Press 1981:139-68.
9. Konturek SJ, Pawlik W. Physiology and pharmacology of prostaglandins. Dig Dis Sci 1986; 31 (Suppl 1):6S-19S.
10. Williams TJ. Interactions between prostaglandins, leukotriens and other mediators of inflammation. Br Med Bull 1983; 39:239-42.
11. Crampton JR, Gibbons LC, Ress WDW. Simultaneous measurement of in vitro gastroduodenal prostaglandin E2 synthesis and degradation in peptic ulcer disease. Scan J Gastroenterol 1987; 22:425-30.
12. Hawkey CJ, Rampton DS. Prostaglandins and the gastrointestinal mucosa: are they important in its function, disease or treatment? Gastroenterology 1985; 89:1162-88.
13. Metha J, Metha P, Ostrowski N. Stimulation of vessel wall, prostacyclin by selective tromboxane synthetase inhibitor OKY-1591. Prostaglandins Leukotrienes Med 1983; 49-52.
14. Schlegel W, Wenk K, Dollinger HC, Raptis S. Concentrations of PGs A, E and F like substances in gastric mucosa of normal subjects and of patients with various gastric disease. Clin Sci 1977; 52, 255-8.
15. Konturek SJ. Prostaglandins in pathophysiology of peptic ulcer disease. Dig Dis Sci 1985; 30 (Suppl):105S-8S.

16. Cohen MM, Cheung G, Lyster DM. Prevention of aspirin - induced faecal blood loss by prostaglandin E2. Gut 1980; 21:602-6.

17. Konturek SJ, Obtulowicz W, Sito E, et al. Distribution of prostaglandins in gastric and duodenal mucosa of healthy subjects and duodenal ulcer patients. Effects of aspirin and paracetamol. Gut 1981; 22:283-9.

18. Pierson RN Jr, Holt PR, Watson RM, Keating RP. Aspirin and gastrointestinal bleeding: Chromate^{51} blood loss studies. Am J Med 1961; 31:259-65.

19. Pemberton RE, Strand LJ. A review of upper-gastrointestinal effects of the newer non-steroidal anti inflammatory agents. Dig Dis Sci 1979; 24:53-64.

20. Dajoni E2, Nissen CH. Gastrointestinal cytoprotective effects of misoprostol: Clinical efficacy overview. Dig Dis Sci 1985; 30 (Suppl): 194-200.

21. Bronski D, Sharon P, Karmeli F, et al. Effect of cimetidine on human gastric and duodenal prostanoid synthesis. Scand J Gastroenterol 1984; 19:457-60.

22. Lee YH, Mollison LA, Cheng WD. The effects of antiulcer agents on indomethacine induced gastric ulceration in the rat. Arch Int Pharmacodyn Ther 1971; 192:370-3.

23. Robert A. An intestinal disease produced experimentally by a prostaglandin deficiency. Gastroenterology 1975; 69:1045-47.