

Duodenal mucosa synthesis of prostaglandins in duodenal ulcer disease

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SUMMARY Synthesis of prostaglandins (PGE₂, PGI₂ and PGF_{2α}) and thromboxane A₂ was investigated in short term incubates of duodenal mucosa biopsies. Mucosa close to the ulcer site synthesised significantly less PGF_{2α} (p<0.001) and PGI₂ (p<0.002) measured as its stable metabolite 6-oxo-PGF_{1α} than healthy mucosa from non-ulcer patients. In paired biopsies taken from the ulcer site and opposite the ulcer in the same patient PGF_{2α} and PGI₂ syntheses were both significantly and similarly depressed when compared with normal mucosa. Synthesis of PGE₂ and TxA₂ (as its stable metabolite TxB₂) was not different in any tissue. There is a defect in the ability of the human duodenal mucosa in duodenal ulcer disease to synthesise PGF_{2α} and PGI₂; the defect is not limited to the ulcer site.

Investigations into the pathogenesis of peptic ulceration and intestinal mucosal injury produced by non-steroidal anti-inflammatory drugs and other damaging agents such as alcohol suggest that the prostaglandins maintain mucosal integrity and accelerate ulcer healing.¹⁻⁴

The mechanisms underlying these beneficial actions are uncertain but studies (mainly with PGE₂) have shown that it can increase mucus⁵⁻⁶ and bicarbonate⁷ secretion. PGE₂ can also inhibit acid secretion,⁴ increase gastric mucosal blood flow⁸ and prevent ion flux and potential difference changes produced by noxious stimuli.⁹⁻¹¹

Despite this substantial knowledge of the capabilities of PGE₂, information of the actual synthesis and content of PGE₂ and other cyclooxygenase derived metabolites (eicosanoids) in gastric and duodenal mucosa of ulcer patients is limited. Moreover, there are several discordant results (see Discussion) and it is probable that extrapolation of results from patients with gastric ulcers will not be valid for duodenal ulceration because of the differences in the types and quantities of prostaglandins synthesised along the gastrointestinal tract. In this study we have examined the synthesis of the prostaglandins (PG), PGE₂, PGF_{2α}, PGI₂ (as its stable metabolite 6-oxo-PGF_{1α}) and

thromboxane A₂ (as its stable metabolite TxB₂) in duodenal mucosa.

Methods

DUODENAL MUCOSAL BIOPSIES

Duodenal mucosa was obtained from 32 subjects with active duodenal ulceration and from 27 subjects undergoing upper gastrointestinal endoscopy for abdominal symptoms later ascribed to irritable bowel. In subjects with duodenal ulcers, the biopsies were taken from within 3 mm of the ulcer and in subjects without ulceration, from the duodenal cap. In 19 patients two biopsies were taken: one close to the ulcer site and another from the duodenal cap directly opposite the ulcer. The biopsies were frozen immediately in liquid nitrogen and stored at -70°C until analysis. No analyses were carried out on fresh tissue and all samples were treated similarly. Subjects taking drugs liable to affect the upper gastrointestinal tract, particularly ulcer healing preparations or non-steroidal anti-inflammatory drugs were excluded from the study.

This study was approved by the Southampton District Ethical Committee and informed consent obtained.

ASSAY PROCEDURES

Biopsy specimens weighing between 1-20 mg (average 4.5 mg) were thawed, weighed, and 2 ml Krebs solution pH 7.5 was added. The solution had

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previously been saturated with 5% carbon dioxide in oxygen and kept at 4°C. Specimens were then pre-incubated at 37°C for 15 minutes and the supernatant discarded in order to minimise measurements of prostaglandins produced by the trauma of the biopsy procedure.

Fresh Krebs (2 ml) was then added and incubated at 37°C for 60 minutes. The supernatant was separated for extraction and the tissue stored at -20°C until protein determination. Approximately 7000 dpm 9-³H prostaglandin F_{2α} (approx 65 pg) was then added as an internal standard to assess procedural losses. The supernatant was acidified with IM citric acid to pH 3-4 and immediately extracted twice by mechanical shaking with three volumes distilled diethyl ether and with centrifugation to separate the aqueous and organic phases. The ether was evaporated to dryness under nitrogen flow at 35°C and the residue reconstituted in 2 ml of 0.1 M phosphate buffer pH 7.5. To assess procedural losses 0.4 ml was taken and its 9-³H PGF_{2α} content measured by scintillation spectrophotometry. The recovery was greater than 95%. Following suitable dilutions PGE₂, PGF_{2α}, 6-oxo-PGF_{1α} (stable metabolite of PGI₂) and TxB₂ (stable metabolite of TxA₂) were measured using selective antibodies which in previous tests had been shown to have low cross reactivities with most other major prostaglandins and their metabolites. PGE₂, however, cross-reacts 46% with PGE₁ at the level required to produce a 50% fall in maximum binding of [³H]PGE₂ to the antibody in the radioimmunoassay.

The PG or TxB₂ content of 0.1 and 0.2 ml of unknown samples was measured and results accepted only if they showed concordance with the standards which were always assessed in parallel. Blank values obtained by passing Krebs solution through the entire procedure were included. In 12 experiments the mean blank values for 0.1 ml of the 2 ml reconstituted extracts were (pg±SD) 3.0±2.1 (PGE₂), 4.0±2.5 (PGF_{2α}), 3.4±2.9 (6-oxo-PGF_{1α}) and 2.3±2.0 (TxB₂). All samples were assayed in duplicate and in four separate samples the PGs and TxB₂ were measured in two consecutive assays to assess reproducibility. The results showed good agreement with no significant difference between estimates in the two assays. Because of the small biopsy samples a more extensive evaluation of the reproducibility of the assays was not possible. In studies in this laboratory, however, on PG synthesis by colonic mucosa and using identical methods, the intra-assay coefficient of variation for PGE₂ was 9.5% (n=8) and for PGF_{2α} was 9.09% (n=7). The respective intraassay coefficients of variation were 9.0% (n=7) and 8.3% (n=7).

Protein concentrations following digestion with 0.1N sodium hydroxide were measured by the method of Lowry *et al.*¹² Results were analysed by Wilcoxon's rank sum test and Student's *t* test (paired or unpaired) where appropriate.

The term synthesis is used in this paper to describe the accumulation of eicosanoids in the incubation medium. 6-oxo-F_{1α} and TxB₂ would not be subject to further metabolic degradation and in the absence of added NAD the metabolism of PGF_{2α} and PGE₂ would be minimal.

Results

Table 1 shows the eicosanoids synthesised by healthy, non-ulcerated mucosa in a group of 27 patients without duodenal ulceration compared with biopsies taken close to the ulcer site in 32 separate patients with duodenal ulceration. During 60 minutes incubation, synthesis of PGF_{2α} and 6-oxo-PGF_{1α} by ulcer tissue was reduced by 45.4% and 47.6% respectively when compared with that synthesised by control mucosa, whereas PGE₂ and TxB₂ showed no differences between control and ulcer subjects.

Table 2 compares eicosanoid synthesis in 19 pairs of mucosal biopsy specimens from the same patients taken close to and opposite the ulcer site. The synthesis of all eicosanoids at both sites was not significantly different. Thus synthesis of PGF_{2α} and 6-oxo-PGF_{1α} in samples taken from opposite the ulcer was also significantly lower than in mucosa from patients without duodenal ulceration by 38.9% and 53% respectively (p<0.01 using Wilcoxon's rank sum test and Student's *t* test).

Discussion

We have shown highly significantly reduced PGI₂ and PGF_{2α} synthesis by duodenal mucosa within 3 mm of ulcer compared with healthy mucosa from

Table 1 *Prostanoid and thromboxane synthesis by duodenal mucosa close to the ulcer site compared with mucosa from the duodenal cap in patients without duodenal ulcer*

	No	PGE ₂	PGF _{2α}	6-oxo-PGF _{1α}	TxB ₂
Control	27	17.6±2.75	10.8±1.07	17.0±2.2	17.4±1.5
Ulcer	32	18.8±2.7	5.9±0.6†	8.9±1.1*	17.9±1.8

Values shown are ng/mg protein ± SEM of eicosanoid released into the incubation fluid in one hour. The values shown in the ulcer row include the 19 samples from close to the ulcer site shown in Table 2. Statistical analysis was performed using Wilcoxon's rank sum test.

* p<0.002 and † p<0.001.

By Student's *t* test * p<0.001 and † p<0.001.

Table 2 Prostanoid and thromboxane synthesis by duodenal mucosa close to the ulcer site and from the duodenal cap directly opposite the ulcer

	No	PGE ₂	PGF _{2α}	6-oxo-PGF _{1α}	TxB ₂
Close to ulcer site	19	14.2±2.4	4.8±0.6	8.0±1.3	17.0±2.15
Opposite ulcer site	19	15.8±3.9	6.6±0.7	8.0±1.3	17.0±2.1

Data shown are paired values (ng/mg protein ± SEM) of eicosanoid released into the incubation fluid in one hour. The eicosanoids synthesised in biopsies from two sites in the same patient were measured in the same assay. Statistical analysis was performed using Wilcoxon's rank sum test and by Student's *t* test.

non-ulcerated patients. Further, the synthesis of eicosanoids in the samples of mucosa from opposite the ulcer site are similarly reduced when compared with mucosa close to the ulcer. It would seem, therefore, that the prostanoid synthesising defect is not just associated with the immediate ulcer area but is a more general duodenal abnormality in ulcer disease and not secondary to the formation of the ulcer.

Comparison of our data with other investigations highlights some dissimilarities. In a similar study to this but utilising different culture conditions Sharon *et al*¹³ showed no reduction in 6-oxo-PGF_{1α} accumulation in culture fluid of incubated duodenal mucosa from ulcer patients; however, like this study they showed no diminished PGE₂ or TxB₂ accumulation. Other investigators also showed no change in PGE₂ synthesis in the duodenum from patients with ulcers compared with healthy duodenal mucosa.¹⁴

Evidence for a more general mucosal defect in eicosanoid synthesis has been suggested¹¹ as seemingly healthy gastric mucosa from patients with duodenal ulceration synthesised less PGE₂, 6-oxo-PGF_{1α} and TxB₂ than gastric mucosa from patients without duodenal disease.¹³ Other investigators, however, could not show this.¹⁴ Further uncertainties exist as other investigators studying tissue content of PGE₂ have shown reduced concentrations in mucosa both at the edge of and 5 cm from the gastric ulcer site compared with normal tissue.¹⁵ Contrary to this, raised concentrations of prostanoids in antral biopsies from gastric ulcer patients (4 cm from the lesion) compared with controls have also been described.¹⁶ These discrepancies may be because of methodological differences or to variations in the site of biopsy. We believe that these may be resolved by more clearly defining the region from which the biopsy is taken and by realising that there will be marked differences between the ability of tissue to newly synthesise prostanoids and thromboxane when compared with the tissue content and, because cells do not contain storage sites for prostaglandins the measurement of tissue content may not be

meaningful.

We were able to show reduced levels of synthesis of only PGI₂ and PGF_{2α} in ulcer patients; this selectivity is perhaps surprising because PGE₂ is also cytoprotective.¹⁷ As PGE₂ and TxA₂ synthesis is maintained in ulcer tissue it is unlikely that there is an overall reduction in cyclooxygenase enzyme activity or substrate availability although we cannot yet be certain of this. The nature of the reduced synthesis suggests that dissimilar cell types may be responsible for the different PGs synthesised and these are altered in ulcer disease. The significance of TxA₂ in peptic ulcer disease is not known; it is vasoconstrictor and platelet aggregatory and as such may exacerbate mucosal damage; however, PGF_{2α} can be vasoconstrictor yet also has cytoprotective properties.¹⁷ The selective reduction in the synthesis of the potent vasodilator PGI₂ while maintaining TxA₂ may lead to reduced blood flow in the ulcerated duodenal mucosa and contribute to the ulcer or delay healing.

It is possible that the relative balance of the PGs and TxA₂ synthesised may be an important factor in the genesis of ulcers as may be the synthesis of the lipoxygenase products of arachidonic acid metabolism.

We conclude that reduced PGI₂ and PGF_{2α} may predispose the duodenal mucosa to injury and ulceration.

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