Duodenal and antral mucosal prostaglandin E₂ synthesis in a study of normal subjects and all stages of duodenal ulcer disease treated by H₂ receptor antagonists

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SUMMARY We tested the hypothesis that the production of prostaglandin E₂ (PGE₂) is impaired in duodenal ulcer disease and affected by treatment and healing. This was investigated by a study of maximal PGE₂ synthesis rates in duodenal and antral mucosal biopsies obtained at endoscopy. The patients were divided into three groups. Group (a): endoscopically normal controls (n=56); group (b): treatment controls (non-DU disease: gastric ulcer or oesophagitis treated by histamine H₂ receptor antagonists) (n=41); and group (c): patients with DU disease (n=183) further subdivided into group (c1) active ulcer not on treatment (n=47), (c2) treated active ulcer (n=35), (c3) healed ulcer on treatment (n=86), and (c4) healed ulcer not on treatment (n=15). Group (a) synthesised (mean (SD)) 106.6 (39.0) pg PGE₂/mg wt of tissue from the duodenal bulb and 129.9 (56.9) from the second part of the duodenum. No difference was found between group (a) and (b) at either site. Group (c1) ulcer rim made 49.8 (22.7) and at all stages ulcer rim and scar made less than the control duodenal bulb (p<0.02). Uninvolved duodenal bulb from groups (c1) (63.4 (31.0)), (c2) (83.6 (38.5)), and (c3) (81.5 (31.1)), however, also made significantly less than controls (p<0.02) and a similar though non-significant trend was seen in group (c4). Biopsies from the second part of the duodenum did not synthesise significantly less than the control group but a similar trend was noticed at each stage of ulcer treatment. Biopsies of control antrum synthesised 124.5 (32.2) but only 93.7 (44.2) in group (c1) (p<0.005). All stages of duodenal ulcer healing were associated with a decreased capacity to synthesise the major prostaglandin PGE₂ at the ulcer site and the uninvolved duodenal bulb and, in acute untreated duodenal ulcer, the uninvolved antrum. This decreased capacity may be the consequence of the disease process itself and not secondary to the treatment, indicating a basic pathophysiological abnormality which may explain the characteristic tendency of the disease to relapse.

Animal work suggests that endogenous prostaglandins may be a component of mucosal protection and rapid repair.¹⁻³ Attempts to estimate mucosal prostaglandin synthesis in patients with peptic ulceration, however, has produced conflicting results in

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gastric and duodenal ulcer.⁴⁻¹³ The majority of studies have looked at relatively few patients and methodological problems may have failed to reveal a consistent pattern.¹⁴ None have looked at synthesis during the healing of peptic ulcers. In this study, therefore, we have looked at mucosal prostaglandin E₂ synthesis in larger groups of patients throughout the healing process using an accurate and reproducible method at two centres. For logistical reasons it was not possible to follow up a cohort of patients

through the different phases of their ulcer disease and instead a cross sectional study design was used.

Methods

PATIENTS

During the gastroscopy clinics of SP and KDB biopsies were taken from the upper gastrointestinal mucosa for the measurement of prostaglandin synthesis. The state of the ulcer disease was routinely monitored by periodic endoscopy during healing and maintenance treatment and in some patients even when off all treatment. Thus, biopsies could be taken at all stages of ulcer disease. The study was approved by the Ethical Committees of University College and Rotherham District Health Authority and all patients gave informed consent. Patients past medical history and social history together with age, sex, and therapeutic history were recorded. All patients with a history of aspirin or other NSAID ingestion in the previous three days was excluded. At gastroscopy the oesophagus, stomach and duodenum to the ampulla were closely examined for ulcers, scars, and inflammation. A patient was judged suitable for use as a control only if he or she had a completely normal investigation and had no past medical history of peptic ulcer disease or treatment with anti-ulcer drugs; biopsies were then taken from the duodenal bulb. Patients with duodenal ulcer disease, past or present, had biopsies of the ulcer rim or of the scar if identifiable, as well as from the duodenal bulb if an area could be found free of associated inflammation. Biopsies were obtained from the second part of the duodenum where possible in both groups. Some controls and patients with untreated duodenal ulcers also had biopsies of endoscopically normal antrum. The patients studied were divided into three groups and are detailed in Table 1.

Table 1 Definition and subdivision of patient groups

Group a	Patient controls; Entirely normal endoscopy, no past medical history of duodenal ulcer or use of anti-ulcer drugs (56 patients).
Group b	Treatment controls; Normal duodenum at endoscopy and no past medical history of duodenal ulcer but currently on H ₂ receptor antagonist for the treatment of gastric ulcer or oesophagitis (41 patients).
Group c1	Active duodenal ulcer at endoscopy, not on any anti-ulcer treatment (47 patients).
c2	Active duodenal ulcer while on H ₂ receptor antagonists, ie not healed (35 patients).
c3	Healed duodenal ulcer at endoscopy but still on treatment with H ₂ receptor antagonists (86 patients).
c4	Healed duodenal ulcer at endoscopy and no longer on H ₂ receptor antagonists (15 patients).

NB In some patients not all areas could be biopsied because of extent of the disease. All treatment was with cimetidine or ranitidine.

BIOPSIES AND METHOD

A modification of the method described by Whittle *et al*¹⁵ was used as indicated below. The biopsies were transferred immediately in ice cold TRIS buffer (pH 8.4) and processed inside one hour. The sample was washed with TRIS buffer, blotted dry on Whatman's no 1 filter paper and weighed using an electronic balance (Metler AC100). The biopsy was then resuspended in 1 ml TRIS buffer and ultra-centrifuged for exactly 15 seconds in a fixed speed Hawksley microhaematocrit centrifuge (giving 10000 rpm). The supernatant was discarded and replaced with 0.05 ml TRIS buffer and the biopsy vortexed for exactly 60 seconds using a bench vortex mixer. The formation of prostaglandins was stopped by the addition of 0.01 ml indomethacin in ethanol (to give a final concentration of 10 µg/ml) and the total volume made up to 1 ml by the addition of 0.94 ml of TRIS buffer. The whole was then recentrifuged as above and the supernatant divided into two aliquots and stored at -20°C. At this stage aliquots from Rotherham were transported in dry ice. Aliquots were batch assayed at University College by highly specific radioimmunoassay for prostaglandin E₂, using an anti-PGE₂ antibody with less than 0.4% cross reactivity with other prostaglandins (Sigma Chemical Co Ltd, Poole, Dorset). The sensitivity of the assay was greatest in the range 10-60 pg/ml which formed the linear portion of the calibration curve. All samples were assayed in duplicate and two dilutions together with reagent and sample blanks. Samples with high prostaglandin levels were further diluted such that they fell within the range of sensitivity. The final results are expressed as picograms PGE₂/mg weight of tissue.

STATISTICAL ANALYSIS

Results were expressed as means (SD) and groups were statistically compared using Student's *t* test for unpaired data. Where *p* values are given for groups of comparisons the least significant value is quoted. Significance was defined at the 5% level.

Results

VALIDATION

Initial validating experiments were carried out and the reproducibility of the method as a whole was assessed using replicated estimations in multiple biopsies obtained from the same site in the same patient at a single gastropscopy. Five patients were studied. More than 95% of the measured PGE₂ was formed during vortexing. Storage of the aliquots at -20°C showed no appreciable decrease in PGE₂ content over a three month period. Results of replicate estimations of six biopsies in each of five

Table 2 Percentage distribution of sex, age, and social habits of control and duodenal ulcer patients

	Control patients		Duodenal ulcer patients	
	Males	Females	Males	Females
Age	50.9	49.1	77.0	23.0
<40		34.6	22.1	12.0
40-60		34.6	52.3	60.0
>60		30.8	25.6	28.0
Social habits				
NS and ND	28.5		11.0	35.7
S but ND	14.2		13.4	21.4
D but NS	23.8		19.5	14.3
S and D	33.5		56.1	28.6

(N)S - (non)smoker. (N)D - (non)drinker.

patients showed good reproducibility with an overall coefficient of variation of 12.1%.

DEMOGRAPHY

The sex ratio, age, and social habits of the duodenal ulcer patients and the controls are detailed in Table 2. Amongst the control patients the sexes were equally represented with similar age distribution and social habits; their results have therefore been combined in this Table. Amongst the duodenal ulcer patients, however, there was a considerable male preponderance (more than three to one). Their age and social

habits also differed and are shown separately. To assess the possible effect of these demographic differences on our results, the PGE_2 synthesis of normal duodenal biopsies from control patients were analysed by sex, age, and social habits (Table 3). No difference were noted. Because of this, the results of the duodenal ulcer patients were analysed as a single group.

PROSTAGLANDIN E_2 SYNTHESIS

The results for prostaglandin E_2 synthesis are detailed in Table 4 and summarised below with all values expressed as pg/mg of tissue.

PATIENT CONTROLS (GROUP (A)) AND TREATMENT CONTROLS (GROUP (B))

The PGE_2 synthesis of biopsies from treatment controls (group (b)) showed no difference from patient control values in either the duodenal bulb or in the second part of duodenum.

DUODENAL ULCER PATIENTS (GROUP (C))

Area immediately adjacent to the ulcer or scar

A decrease in PGE_2 synthesis was evident in groups (c1) (49.8 (22.7)), (c2) (65.9 (35.3)), (c3) (68.9 (36.1)), and (c4) (74.2 (21.1)) compared with the control duodenal bulb (106.6 (39.0)). This difference was significant for all stages (p values <0.02) but was most marked when the ulcer was active.

Table 3 The effect of sex, age, and social habits on PGE_2 (pg/mg weight of biopsy) synthesis in normal duodenal mucosa

	Both sexes							
	Age range (years)*					Drinking and smoking†		
	Male	Female	<40	40-60	>60	NS+ND	S+ND	D+NS
Mean	99.7	109.1	105.3	103.2	108.1	99.2	93.7	96.2
SD	28.6	30.9	29.0	30.2	31.9	21.2	12.9	25.0
N	27	26	17	17	15	12	6	10

*None of the age groups differed significantly from each other; †None of the social habits groups differed significantly from each other.

Table 4 Mean (SD) [n] duodenal prostaglandin E_2 synthesis (pg of PGE_2 /mg weight of tissue) by site and disease stage; all ulcers treated by H_2 receptor antagonists

Groups	Ulcer rim or scar	Uninvolved bulb	Second part
Control (a)	-	106.6 (39.0) [56]	129.9 (56.9) [12]
Active ulcer (c1)	49.8 (22.7) [47]*	63.4 (31.0) [34]*	105.8 (90.7) [15]†
Treated ulcer (c2)	65.9 (35.3) [35]*	83.6 (38.5) [32]*	96.1 (36.9) [14]†
Healed on rx (c3)	68.9 (36.1) [16]*	81.5 (31.1) [86]*	99.8 (52.9) [36]†
Healed off rx (c4)	74.2 (21.1) [10]*	91.2 (39.8) [15]†	103.1 (57.9) [6]†
Non-DU on rx (b)	-	108.5 (38.9) [41]‡	120.7 (25.1) [6]‡

rx = Treatment. *Less than normal duodenal bulb (p<0.02); †Disease associated duodenal bulb or second part of duodenum not different from control; ‡Treated non-duodenal ulcer duodenal bulb and second part not different from normal.

Uninvolved bulb

PGE₂ synthesis was also reduced in group (c1) (63.4 (31.0)), (c2) (83.6 (38.5)), and (c3) (81.5 (31.1)) compared with the control group duodenal bulb (p values <0.02). The differences were less marked than those seen adjacent to the ulcer or scar. Group (c2) patients with unhealed ulcer but on H₂ receptor antagonists synthesised more PGE₂ adjacent to the ulcer and in the uninvolved bulb than group (c1) patients with active but untreated ulcer (p values <0.05).

Second part of the duodenum

There appeared to be a trend towards a reduction in PGE₂ synthesis but this did not achieve statistical significance. In patients and control groups the second part of the duodenum synthesised more PGE₂ than the bulb (p values <0.02).

Antrum

In the control group PGE₂ synthesis (124.5 (32.1)) was significantly higher (p<0.003) than the uninvolved antrum of group (c1) (93.7 (44.2)). In controls and DU patients antral PGE₂ synthesis was greater than that in the duodenal bulb (p values 0.02).

Discussion

These results show that there is a deficiency in the ability of the duodenal mucosa near to and remote from an active duodenal ulcer, both before and during treatment with H₂ receptor antagonists, to synthesise prostaglandin E₂. This deficiency persists at the ulcer site after healing and cessation of treatment. In the duodenal bulb away from the ulcer and assessed to be normal at endoscopy a similar, parallel, but less severe deficiency is seen. This changing pattern of PGE₂ synthesis in the duodenal bulb may be an effect of treatment or of healing or both. It seems unlikely to be the result of treatment alone for no such change was observed in the treatment control group. The healing process may contribute but this is unlikely to be the full explanation, as PGE₂ synthesis does not quite attain normal values (albeit it is not significantly lower), neither at the ulcer site nor remote from it, despite complete endoscopic healing. A third possibility is that the cross sectional design of the study may have favoured selection of patients with milder disease (ie less likely to relapse) into the later groups (c3) and (c4). Certainly a high percentage of duodenal ulcer patients healed by H₂ receptor antagonists relapse within a year off treatment.¹⁶ Thus our later groups may contain a higher proportion of patients who are resistant to relapse than our earlier groups ((c1) and

(c2)). If endogenous prostaglandin production is important in maintenance of healing then those patients with lower rates of synthesis will relapse early and only those patients with relatively higher synthesis rate will be left in remission. This will have the effect of raising the mean PGE₂ values of the later groups. Our study which does not follow individual patients through the different stages of their ulcer disease cannot confirm or refute this theory.

Antral biopsies from patients with active untreated ulcer (group (C1)) showed a significant reduction in synthesis ability compared with those from control patients (group (a)). The finding of a defect in PGE₂ synthesis so remote from the ulcer site suggests that this is a true primary biochemical abnormality and not secondary to inflammation or scarring in the disease process.

We have shown that duodenal PGE₂ production is impaired in DU disease and for the first time it has been shown that a deficiency persists after healing and also exists in the uninvolved duodenal bulb and antrum. Our results, however, are different from those of other workers. For example, Sharon *et al*⁹ using an organ culture method, found no difference in duodenal biopsies from patients with active DU and normal controls, in the accumulations of PGE₂, 6 keto PGE₂ and thromboxane B₂. Hillier *et al*⁵ also using a culture method noted significant reductions in PGF_{2α} and PGI₂ synthesis but not of PGE₂ or thromboxane B₂ in active DU compared with healthy controls. Crompton *et al*⁸ used homogenised duodenal biopsies to which a solution containing various cofactors, including the prostaglandin substrate arachidonic acid, was added; they observed no difference in PGE₂ synthesis between DU patients and normals. In contrast, our method in which tissue samples are vortexed, measures the maximal PGE₂ synthesis rate from endogenous substrate. These differences in results may well be the consequence of methodological differences in assays (at least in part) which because of the nature of prostaglandins, have a crucial bearing on the outcome. This makes it difficult to compare results when different methods are used.¹⁴

The pathophysiology of duodenal ulcer has generally been viewed as an imbalance between 'attack factors' and 'mucosal defence'. Acid and pepsin secretion are the principal components of the 'attack factors' and have been extensively studied whereas until recently, methodological problems have restricted studies in mucosal defence abnormalities. Advances have, however, now been made. Isenberg *et al*¹⁷ have shown reduced bicarbonate secretion in DU patients and Quigley and Turnberg¹⁸ have shown that DU patients have an impaired ability to maintain the juxtamucosal neutral zone in the face of luminal

acid. These pathophysiological abnormalities may in turn be associated with the impaired PGE₂ production we have demonstrated.

If, as this study suggests, there is a deficiency in the synthesis of PGE₂ *in vivo* in patients with duodenal ulcer before and after treatment with H₂ antagonists, this may explain the high relapse rate after such treatment and because this deficiency is not limited to the ulcer site explains why the ulcer may relapse in other parts of the duodenal bulb.

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