Eicosanoid synthesis in duodenal ulcer disease: decrease in leukotriene C₄ by colloidal bismuth subcitrate

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Abstract

The release of immunoreactive prostaglandin E₂ (PGE₂) and leukotriene C₄ (LTC₄) from antral and duodenal mucosal biopsy specimens taken from 20 patients with duodenal ulcer disease was measured by radioimmunoassay before and four weeks after treatment with colloidal bismuth subcitrate. Gastroscopic and histological examination showed complete ulcer healing in 15/18 patients and duodenal histology looked normal (n=15) or improved (n=3): two patients failed to attend for a second endoscopy. Analysis of the supernatant from incubations of biopsy tissue *in vitro* showed that unstimulated antral release of PGE₂ was significantly more than that from the duodenal mucosa (p<0.05), whereas basal release of LTC₄ was significantly lower from antral biopsy specimens (p<0.05). Subsequent incubation of specimens with calcium ionophore A23187 caused an increase in LTC₄ but not in PGE₂ generation. The ability of antral and duodenal mucosa to form ionophore mediated LTC₄ in patients with duodenal ulcer disease was significantly greater (p<0.05; p<0.01 respectively) than that of normal gastroduodenal mucosa. After colloidal bismuth subcitrate treatment, basal synthesis of PGE₂ was unchanged in duodenal and antral specimens. In contrast, basal duodenal LTC₄ was reduced (p<0.05), and the capacity for ionophore mediated duodenal LTC₄ formation was substantially and significantly reduced after treatment (p<0.001). These results indicate that after therapeutic healing of duodenal ulcer (accompanied by clearance of inflammatory cell infiltrate), there is a reduced ability of duodenal mucosa to generate proinflammatory peptidoleukotrienes.

The role of prostaglandins in the pathogenesis of duodenal ulcer disease remains controversial, and the importance of the vasoconstrictor and proinflammatory peptidoleukotriene LTC₄ is not fully established. Early reports showed that exogenous LTC₄ exerts a powerful influence on the rat gastric microcirculation leading to a reduction in mucosal blood flow,¹ and that mucosal generation of LTC₄ in rats was increased considerably after ethanol administration.² The initial findings of Peskar *et al*² and Wallace *et al*³ that inhibitors of peptidoleukotriene synthesis and the dual cyclo-oxygenase/5-lipoxygenase inhibitor BW 755C offer appreciable protection against ethanol induced damage in rats remains controversial. Boughton-Smith *et al*, however, have shown that the selective 5-

lipoxygenase inhibitor BWA4C affords partial protection against platelet activating factor (PAF) induced gastric damage.⁴ Moreover, infusion of LTC₄ increases the susceptibility of the rat stomach to injury by ethanol.⁵

Colloidal bismuth subcitrate is known to be effective in promoting duodenal ulcer healing in man⁶ and seems to offer the prospect of a lower rate of relapse than follows successful treatment with histamine H₂-receptor antagonists.^{7,8} Although the mechanism of these effects has not been fully explained, animal studies show that colloidal bismuth subcitrate is protective against ethanol induced gastric lesions and that this is associated with increased mucosal generation of prostaglandin E₂ (PGE₂).^{9,10} Recent studies have shown that colloidal bismuth subcitrate provides partial protection from aspirin induced lesions despite concomitant suppression of prostaglandin biosynthesis.^{11,12} This suggests that it may confer protection by mechanisms other than by increasing prostaglandin production.

There is a need for further investigation of the relation between colloidal bismuth subcitrate, ulcer healing, and arachidonate metabolism in man. In this study the release of immunoreactive LTC₄ and PGE₂ from human gastroduodenal mucosal biopsy tissues was measured *ex vivo* in patients with duodenal ulcer disease both before and four weeks after a successful course of colloidal bismuth subcitrate treatment.

Methods

PATIENTS

Twenty patients (15 men and five women), mean age 46 (range 19-77) years with an endoscopically proved duodenal ulcer and 15 subjects with upper abdominal pain who were found to be endoscopically normal (12 men and three women), mean age 42 (range 22-69) years were studied. Endoscopic biopsy specimens were taken before treatment and, in the case of the duodenal ulcer disease patients, after four weeks' treatment with colloidal bismuth subcitrate (240 mg given twice daily). Twenty four hours before the second endoscopy, all patients had stopped colloidal bismuth subcitrate treatment. Multiple biopsy specimens were obtained from the lesser curve of the gastric antrum and duodenal bulb at 5 mm from the ulcer rim. One of the specimens was taken for histological analysis, performed by an observer without knowledge of the biochemical results, and graded as uninfamed (normal) or infamed (moderate or considerable increase in inflammatory cell infiltrate) using standard histological criteria.¹³

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This study was approved by the Clinical Investigation Panel at The Middlesex Hospital and written informed consent was obtained from all subjects.

PREPARATION AND INCUBATION OF BIOPSY SPECIMENS

Preparation and incubation procedures were based on those described by Dreyling *et al*, with minor modifications.¹⁴ The organ culture method in this study employs unstimulated culture for a fixed period followed by a chemically challenged incubation for an identical period. Eicosanoids released during culture were collected from the bathing medium and measured by radioimmunoassay.

Five biopsy specimens for each incubation tube were immediately pooled and washed in 0.5 ml ice cold 0.9% saline. The pooled biopsy specimens (mean (SEM) 18.5 (2.3) mg wet weight antrum and 16.3 (3.2) mg wet weight duodenum) were placed in 0.5 ml 0.9% saline at 4°C for 20 minutes to facilitate the leaching out of any eicosanoids generated during the procedures, and the supernatant was discarded.

After the washout period, the group of five duodenal and antral biopsy specimens was transferred to a similar set of tubes containing 0.5 ml prewarmed and oxygenated (95% O₂+5% CO₂) modified Tyrode solution (composition, g/l: NaCl 8.0, KCl 0.2, MgCl₂.6H₂O 0.21, NaHCO₃ 1.0, NaH₂PO₄.H₂O 0.058, CaCl₂.2H₂O 0.13 glucose 1.1) and incubated in a water bath at 37°C for 20 minutes under continued oxygenation ('basal release').

Biopsy specimens were transferred immediately into a further set of tubes containing fresh prewarmed/oxygenated buffer containing 1 µmol/l of Ca²⁺ ionophore A23187 (Sigma Chemical Company Ltd). Incubation was continued in the presence of the ionophore for a further 20 minutes at 37°C ('ionophore mediated') and the reaction was terminated by complete removal of the incubation medium. Calcium ionophore A23187 elicits an increase in intracellular Ca²⁺ which initiates activation of the arachidonic acid cascade. After this incubation the pooled biopsy specimens were removed and weighed and the supernatants from both sets of incubations stored at -20°C. In some experiments (n=8), as indicated, the second incubation was performed in the absence of ionophore A23187.

RADIOIMMUNOASSAY

Supernatants from both basal and stimulated release were stored at -20°C for radioimmunoassay - usually within four weeks. Aliquots (25

µl of sample) were assayed in triplicate for PGE₂ without extraction using antibodies and double antibody precipitation procedures as described by Berry *et al*,¹⁵ and the assay sensitivity was 15 pg/tube. LTC₄ was measured by a specific LTC₄ radioimmunoassay kit (New England Nuclear, DuPont, UK). The antibodies used for determination of the peptidoleukotriene had cross reactivities of 100% with LTC₄ and 11.6% with LTD₄, the assay sensitivity was 20 pg/tube, and the substance(s) detected using this assay are referred to throughout this paper as 'immuno-reactive LTC₄'. The intra-assay and the inter-assay coefficients of variation for the PGE₂ and LTC₄/LTD₄ radioimmunoassays were 11.7% and 12.3% (PGE₂) and 9.9% and 15.7% (LTC₄)

STATISTICS

PGE₂ and LTC₄ values are shown as mean (SEM) expressed as pg/mg wet weight/20 minute incubation (pg/mg/20 minutes). Paired and unpaired data were evaluated using the Student's *t* test.

Results

MACROSCOPIC AND HISTOLOGICAL FINDINGS

In all patients with duodenal ulcer disease, histological examination of the antrum before therapy showed active chronic gastritis with an inflammatory cell infiltrate of predominantly neutrophils and polymorphs. The duodenum showed mild to moderate inflammation in 17 patients, while three patients had normal duodenal histology. Four weeks after treatment with colloidal bismuth subcitrate, gastroscopy showed complete ulcer healing in 15/18 patients. Two patients failed to attend for a second gastroscopy. Antral and duodenal histology returned to normal in 18 and 15 patients respectively after treatment. Control subjects had normal antral and duodenal histology as well as being endoscopically normal.

LTC₄/PGE₂ RELEASE IN DUODENAL ULCER DISEASE BIOPSY SPECIMENS BEFORE TREATMENT

Immunoreactive PGE₂ and LTC₄ were released from the biopsy specimens into the bathing media when incubated at 37°C for 20 minutes (basal condition). The mean (SEM) antral basal release of PGE₂ was 1438.0 (176.0) pg/mg/20 minutes, and was significantly greater than the mean (SEM) duodenal basal release of 1029.0 (122.0) pg/mg/20 minutes (p<0.05) (Fig 1A). The release of LTC₄ was approximately 20 fold less in the antrum and 10 fold less in the duodenum than that of PGE₂. The mean (SEM) basal release of LTC₄ from antral biopsy specimens was 58.7 (9.5) pg/mg/20 minutes, and was significantly lower than from duodenal specimens (95.5 (13.7) pg/mg/20 minutes (p<0.005) (Fig 1B)).

The generation of LTC₄ was increased compared with basal release for both antral and duodenal mucosal tissues when they were transferred for a second incubation with Ca²⁺ ionophore. Again LTC₄ generation was

Effect of ionophore A23187 on eicosanoid release. (Values mean (SEM) of eight experiments)

	LTC ₄ (pg/mg/20 min)		PGE ₂ (pg/mg/20 min)	
	Antrum	Duodenum	Antrum	Duodenum
Basal (first incubation)	42 (11.1)	82 (14.2)	1206 (210)	934 (103)
+ A23187 (second incubation)	94 (29.7)	197 (32.4)	348 (52.9)	220 (44.2)
- A23187 (second incubation)	36 (9.8)	65 (12.9)	329 (44.6)	243 (39.3)

LTC₄=leukotriene C₄; PGE₂=prostaglandin E₂.

Figure 1: Release of immunoreactive prostaglandin E₂ (A) and leukotriene C₄ (B) from human mucosal biopsy specimens before and after treatment with colloidal bismuth subcitrate. Data show mean (SEM) values for antral and duodenal biopsy specimens incubated under basal conditions or after transfer to fresh buffer containing 1 μ mol/l A23187. The significances of differences between different anatomical sites or between basal and ionophore mediated release are described in the text.

significantly lower in the antral mucosa than in duodenum, but in both cases output was significantly greater in the A23187 incubation than under basal conditions (antrum: 88.0 (15.2) pg/mg/20 minutes, $p < 0.05$; duodenum: 171.0 (22.7) pg/mg/20 minutes, $p < 0.01$) (Fig 1B).

In contrast, the release of PGE₂ from antral and duodenal mucosal biopsy specimens was not stimulated by the ionophore A23187, but was significantly reduced (antrum: 436.0 (8.2) pg/mg/20 minutes ($p < 0.001$); duodenum: 385.0 (69.7) pg/mg/20 minutes ($p < 0.001$)) compared with the basal PGE₂ release (Fig 1A).

To examine the effect of A23187 further, experiments were performed comparing the effect of a second incubation with A23187 with that of a second incubation using buffer alone (Table). In this experiment, the amounts of LTC₄ and PGE₂ and the differences between the antrum and duodenum were very similar to those described above (cf Fig 1). The amount of PGE₂ released after addition of the ionophore A23187 did not differ significantly from the amount released during an identical incubation period in the absence of ionophore. The result indicates that PGE₂ synthesis seems to be Ca²⁺ independent in this system. On the other hand and as expected, the release of LTC₄ was increased by the calcium ionophore but not in its absence (Table).

The generation of eicosanoids by biopsy samples taken from subjects ($n = 15$) with histologically normal tissue was studied. The release of PGE₂ into the bathing medium from normal antral and duodenal biopsy fragments was similar in the order of magnitude and profile to that detected in gastroduodenal mucosa from patients with duodenal ulcer disease (data not shown). The mean (SEM) basal release of LTC₄ was 63.6 (9.6) pg/mg/20 minutes from normal antral mucosa and 140.0 (15.1) pg/mg/20 minutes from normal duodenal mucosa. The unstimulated mean LTC₄ value from normal antral mucosa was similar in value to the basal

antral release from duodenal ulcer disease mucosa (58.7 (9.5) pg/mg/20 minutes), but, surprisingly, the basal duodenal LTC₄ release from normal mucosa was significantly ($p < 0.05$) raised compared with the unstimulated release from duodenal ulcer disease mucosa (95.5 (13.7) pg/mg/20 minutes). Analysis of ionophore mediated LTC₄ formation showed an interesting difference between normal and abnormal mucosa in the ability of the ionophore to induce LTC₄ generation. This was significantly reduced in normal antral ($p < 0.05$) and duodenal ($p < 0.01$) mucosa compared with gastroduodenal mucosa from duodenal ulcer disease patients (Fig 2).

LTC₄/PGE₂ RELEASE IN DUODENAL ULCER DISEASE BIOPSY SPECIMENS AFTER TREATMENT

No significant difference was found in PGE₂ release from antral and duodenal biopsy specimens before and after treatment with colloidal bismuth subcitrate (Fig 1A). This was true for comparisons of basal release and for release in the presence of the ionophore.

Analysis of LTC₄ formation in biopsy specimens taken from patients after treatment showed considerable differences from the pretreatment values. In antral tissue obtained after completion of colloidal bismuth subcitrate treatment ($n = 18$), there was a significant reduction in basal ($p < 0.02$) but not in ionophore mediated LTC₄ release compared with tissue samples taken before treatment (antral basal value 39.0 (5.9) pg/mg/20 minutes; antral stimulated value 74.0 (13.9) pg/mg/20 minutes) (Fig 1B). The basal duodenal LTC₄ release was reduced after treatment compared with basal values before therapy ($p < 0.05$) (Fig 1B). More interestingly, the ability of the ionophore to stimulate the release of LTC₄ from duodenal mucosa was substantially and significantly reduced after treatment compared with beforehand ($p < 0.001$). The mean ionophore mediated LTC₄ value fell from 171.0

Figure 2: Ionophore mediated leukotriene C_4 formation from antral and duodenal mucosa of normal subjects and duodenal ulcer (DU) disease patients. Data are expressed as a percentage of unstimulated (basal) release, where basal release is shown as 100%.

(22.7) before treatment to 94.5 (11.4) pg/mg/20 minutes after treatment with colloidal bismuth subcitrate (Fig 1B).

Discussion

This study shows for the first time the capacity of gastric antral and duodenal mucosal specimens from patients with duodenal ulcer disease to synthesise immunoreactive LTC_4 . In addition, the results show that ulcer healing after treatment with colloidal bismuth subcitrate is accompanied by a reduction in the capacity to synthesise LTC_4 , particularly in respect of duodenal mucosa. Decreased ionophore mediated release of LTC_4 may result either from the process of ulcer healing or the effect of colloidal bismuth subcitrate, or may be a combined effect. Synthesis and metabolism of peptidoleukotrienes from 'normal' mucosa has so far been described only in human gastric mucosa from patients with gastric cancer,¹⁴ and the importance of peptidoleukotrienes in duodenal ulcer disease is unknown.

Topical application of LTC_4 is known to cause intense vasoconstriction of submucosal venules, leading to reduced mucosal blood flow¹ which is considered to be a major contributory factor in the pathogenesis of peptic ulceration.¹⁶ Initial findings that the extent of ethanol induced damage in the rat *in vivo* correlated with mucosal LTC_4 formation *in vitro*² and that pretreatment with inhibitors of peptidoleukotriene synthesis caused a parallel decrease in the ulcer index and LTC_4 formation² suggested that LTC_4 may mediate the vasocongestion that precedes ulceration induced by ethanol. Boughton-Smith *et al*, however, pretreated rats with specific 5-lipoxygenase inhibitors and showed that gastric haemorrhagic damage induced by 100% ethanol occurred despite inhibition of leukotriene synthesis¹⁷ arguing against leukotrienes as primary agents of injury in this acute model. More recently, using platelet aggregating factor

as the damaging agent, the same group has shown that an increase in mucosal LTC_4 formation contributes to gastric damage in the rat.⁴

The relevance of these findings in rats to chronic relapsing ulcerative disease in man is unclear. Our data showing reduced ionophore mediated release of gastroduodenal mucosa after ulcer healing are consistent with the view that LTC_4 is an important inflammatory mediator, although we imagine that it is probably not the primary agent of injury, but rather a secondary mediator which may amplify mucosal injury. This view is supported by the results shown in Figure 2, which indicate that the ability of the mucosa to generate LTC_4 is greater in duodenal ulcer disease mucosa than in normal mucosa. These results are supported by an earlier report suggesting that normal uninflamed gastric mucosa generates significantly less ionophore mediated LTC_4 than inflamed gastric mucosa.¹⁸

A fundamental problem in assessing the biological importance of eicosanoids is that any perturbation of cell membranes will initiate eicosanoid synthesis, which means that the sampling procedure *per se* usually stimulates the synthesis and possibly changes the profile of arachidonic acid metabolites. In this study, steps were taken to minimise the effect of sampling trauma by discarding the first collection. The formation of various cyclo-oxygenase products of arachidonic acid metabolism, including PGE_2 , by normal and duodenal ulcer disease gastroduodenal mucosa has been described previously.^{19, 22} The present study, however, shows important differences between the 5-lipoxygenase and the cyclo-oxygenase pathways in the gastroduodenal mucosal biopsy specimens of duodenal ulcer patients. Under basal conditions, the release of PGE_2 from both the antrum and the duodenum was considerably higher than the release of LTC_4 . The observed differences in the levels of these two eicosanoids are probably due to differences in the mucosal cyclo-oxygenase and lipoxygenase enzyme activity. Human antral and duodenal mucosa generate significantly larger quantities of PGE_2 than LTC_4 , probably because of higher cyclo-oxygenase activity in these tissues. Important regional differences in the values of these two eicosanoids were also noted; basal synthesis of PGE_2 was greater in antral mucosa than in duodenal fragments, whereas LTC_4 formation was greater in duodenal than in antral mucosal tissue. These findings are consistent with an earlier report on the prostanoid profile in the human gastrointestinal tract.²⁴

The calcium ionophore A23187 stimulated mucosal LTC_4 formation but had no effect on mucosal PGE_2 synthesis. It has been suggested that the prostaglandins and leukotrienes that can be shown to be released from specimens of mucosal tissue may come from different cellular sources.²⁴ It is also possible that the inability of calcium ionophore to stimulate mucosal PGE_2 formation may be related to a Ca^{2+} independent phospholipase A_2 as the rate limiting enzyme that catalyses the hydrolysis of phospholipids containing arachidonic acid. Several recent studies have raised the possibility that phospholipase A_2 activation also involves Ca^{2+} independent mechanisms.²⁵⁻²⁷ The presence of a Ca^{2+}

independent phospholipase A₂ with a substrate specificity for alkyl containing glycerophospholipids was shown in fetal rabbit lung tissue³⁵ and human amnion tissue.³⁶ It has also been shown that a phosphatidylethanolamine specific phospholipase A₂ purified from human platelets does not require Ca²⁺ for activity.³⁷

Colloidal bismuth subcitrate was found to be effective in healing duodenal ulcers, and this is consistent with previous reports.^{6,7} In contrast with earlier workers,^{9,10} who showed increased PGE₂ synthesis after intragastric administration of colloidal bismuth subcitrate in rats, however, no change in the PGE₂ profile was found before and after colloidal bismuth subcitrate treatment. One possible explanation for this difference may be that in the acute ethanol induced rat model, topical application of colloidal bismuth subcitrate may act as a mild stimulant, thus generating endogenous PGE₂ via the mechanism commonly known as adaptive cytoprotection. No such mechanism, however, would be operative in chronic duodenal ulcer disease.

In this study, treatment of duodenal ulcers with colloidal bismuth subcitrate was associated with the clearance of inflammatory cell infiltrate as well as with a decreased synthesis of LTC₄ after ionophore stimulation. These results suggest that decreased LTC₄ formation is probably a secondary event caused by the reduction in the numbers of inflammatory cells and that the peptidoleukotrienes are probably not the primary agents of injury but may play a role in the development ulceration

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