Prostaglandins Leukotrienes and Essential Fatty Acids (1991) 43, 55–59

15-HETE is the Main Eicosanoid Present in Mucus of Ulcerative Proctocolitis

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ABSTRACT. **Prostaglandins, leukotrienes and mono-hydroxy acid products of arachidonic acid were measured in mucus of freshly recovered morning stools of a patient with an exacerbation of ulcerative proctocolitis. Eicosanoids in ether extracts were separated by high performance liquid chromatography and amounts determined by radioimmunoassay. Four hydroxy-eicosatetraenoic acids were detected, of which the most important one was identified as 15-hydroxy eicosatetraenoic acid (530 ng/g mucus). Leukotriene B_4 was also present (21 ng/g mucus) and small amounts of immunoreactive leukotriene C_4 (< 0.8 ng/g mucus). The prostaglandins 6-keto-$PGF_{1\alpha}$ and PGE_2 and thromboxane B_2 were found in amounts of 3.7, 2.0 and 9.2 ng/g mucus, respectively.**

INTRODUCTION

It has been suggested that lipoxygenase products play a role in bowel disease and in particular in ulcerative colitis (1, 2). In inflammatory bowel disease, the synthesis of leukotriene B_4 (LTB_4) in colonic mucosa has been shown to be enhanced compared to normal tissue (3). Using equilibrium dialysis of the rectum Lauritsen et al found increased concentration of prostaglandin E_2 and $F_{2\alpha}$ and thromboxane B_2 in rectal dialysates of patients with ulcerative colitis, Crohn's colitis and Clostridium difficile colitis, and substantially increased levels of leukotriene B_4 in patients with ulcerative colitis (4, 5). Effects of prednisolone and 5-aminosalicylic acid on these LTB_4 levels were investigated as well as proven selective 5-lipoxygenase inhibitors (6). In these in vivo investigations a significant decrease of LTB_4 was observed, however the clinical efficacy still must be shown in controlled trials (7).

In several animal models of inflammatory bowel disease effects of leukotriene synthesis inhibitors is investigated. Acute and chronic models have been developed in rats (8), rabbits (9) and mice (10). Most studies were focussed on the synthesis or measurements of levels of LTB_4 and PGE_2. Recently more evidence for the involvement of other 5-lipoxygenase products, the sulfidopeptide-leukotrienes, was found (11, 12). Studies in which the metabolism of exogenous arachidonic acid in colonic mucosa in inflammatory bowel disease was investigated, also showed a 3–5-fold increase of other eicosanoids, such as 12- and 15-HETE and HHT (3). It is well known that mono-HETEs act as mediators in mucus secretion (13) and have inflammatory effects in skin (14).

In the present study, lipoxygenase products were determined in intestinal mucus from an individual with mild ulcerative proctocolitis. Mucus secreted with stool was collected from fresh morning stools for 4 days, separated and extracted with diethylether. The eicosanoids were determined and identified by high performance liquid chromatography (HPLC) and radioimmunoassay (RIA). High levels of another lipoxygenase product, 15-HETE, were found in addition to LTB_4.

MATERIALS AND METHODS

Patient history

The patient was a 35-year-old man. He started to lose blood with his stools when he was 15. After

Date received 6 October 1990
Date accepted 3 January 1991

2 years of complaints he was admitted to hospital when fiberoptic sigmoidoscopy revealed a friable mucosa, frank bleeding and some pseudopolyps. The histology was compatible with ulcerative colitis and a diagnosis of ulcerative proctocolitis was made. A remission was induced with prednisone and sulfasalazine. Several recurrences occurred which responded to prednisone and sulfasalazine. Ten years thereafter colonoscopy again revealed friable mucosa in the rectum and sigmoid with normal mucosa in the transverse and descending colon. Treatment with prednisone was again instituted after a rash developed on sulfasalazine. The steroids were gradually tapered off. Since then the patient has not been on drug therapy. A fiberoptic sigmoidoscopy showed a very mild proctocolitis which was confirmed by histology. At the time of the study he was feeling well, had no fever and produced a stool of normal consistence surrounded by mucus, once daily (15).

Methods

Morning stools were collected and mucus separated immediately. Extraction was performed with diethylether (5 ml per gram mucus). Tritiated prostaglandins (PGs), leukotrienes (LTs) and mono-hydroxy-eicosatetraenoic acids (HETEs) were added to measure recoveries. After centrifugation (5 min, 2800xg) the supernatant was collected and evaporated under nitrogen at 40°C. The extract was dissolved in 300 ml of HPLC solvent system and filtered over an Acro C3A 0.45 mm filter (Gelman). The sample was kept in a plastic micro vial (Weichmann, Switzerland). One hundred ml was injected into a ChromSep Nucleosil $5C_{18}$ column (Chrompack, Middelburg, The Netherlands) using a 1084B high performance liquid chromatograph of Hewlett Packard. The solvent system contained: tetrahydrofuran-methanol-0.1% EDTA in water-acetic acid (25:30:45:0.1) adjusted to pH 5.5 with ammonium hydroxide. The flow rate was 0.4 ml/min, oven temperature 37°C. Absorption was monitored as indicated in the Figure. Fractions of 1 per min were collected by a LKB Superrac. Twenty-five ml portions were used for RIA of LTs and HEs. PGs which coelute with LTC_4 were measured by RIA of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), thromboxane B_2 (TxB_2) and prostaglandin E_2 (PGE_2), for which purposes 25 ml of the fractions 4–8 were used. The method was described earlier, in detail (16).

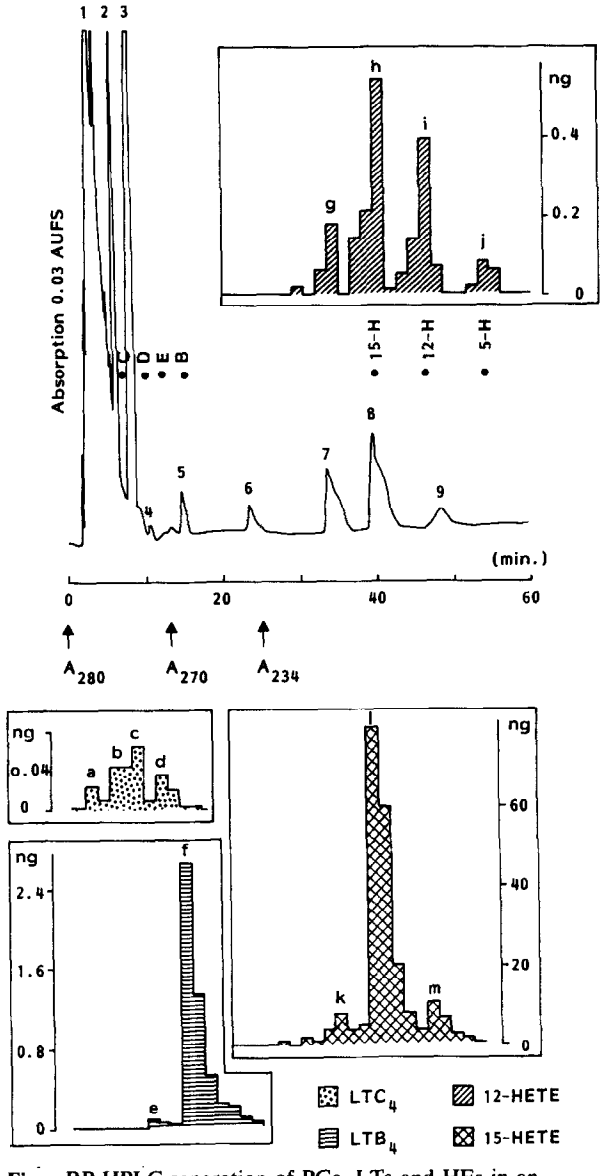

Fig. RP-HPLC separation of PGs, LTs and HEs in an extracted mucus sample of a patient with ulcerative procto colitis. A ChromSep Nucleosil $5C_{18}$ column (200 × 3.0 mm) was used. Mobile phase: tetrahydrofuran-methanol-0.1% EDTA in water-acetic acid (25:30:45:0.1) adjusted to pH 5.5 with ammonium hydroxide. The flow-rate was 0.9 ml/min, oven temperature 37°C.

Explanation of figures: standards (•) C, D, E and B: t_R of synthetic LTC_4, D_4, E_4 and B_4 respectively; standards (•) 15-H, 12-H and 5-H: 15-HETE, 12-HETE and 5-HETE, respectively.

Identification of peaks (as indicated in Results): 1 = front, 2,3 = tri-HETEs and lipoxins, 4 = LTD_4, 5 = LTB_4 (5-(S),12-(R)-dihydroxy-6,8,10,14-eicosatetraenoic acid), 6 = 5,12- and 8,15-diHETE, 7 = unknown HETE, 8 = 15-HETE, 9 = 8- and/or 11-HETE.

From time 0 absorption was monitored at 280 nm, at time 13 at 270 nm and at 25 min at 234 nm. Fractions of 1 per min were collected from 0–18 min for RIA determinations of LTC_4 (▩), from 0–23 min for immunoassay of LTB_4 (▤), from 20–60 min for immunoassay of 12-HETE (▨) and from 20–53 min for the assay of 15-HETE (▩). Explanation of peaks a–m are given in the Results section.
In fractions 4–8 PGs were measured (data shown in the Table)

Chemicals

Synthetic LTs were generous gifts of Dr. J. Rokach (Merck Frosst Labs, Canada), mono-HETEs were obtained from Seragen (Boston, MA, USA) as well as anti-12-HETE, anti-15-HETE and anti-6-keto-$PGF_{1\alpha}$ antibodies. Standards of 6-keto-$PGF_{1\alpha}$, TxB_2 and PGE_2 were purchased from Sigma (USA), and

anti-TxB_2 and anti-PGE_2 from l'Institut Pasteur (Paris, France). Anti-LTB_4 was obtained from Wellcome Research (UK) and a LTC_4 RIA kit from Dupont de Nemours (NEN Division, Germany).

The cross reactivities (> 0.1%) at 50% B/B_0 were as follows: anti-6kPGF$_{1\alpha}$: $PGF_{1\alpha}$ 7.8%, $6kPGE_1$ 6.8%, $PGF_{2\alpha}$ 2.2%, PGE_2 0.6%; anti-TxB_2: all < 0.1%; anti-PGE_2: $kPGE_2$ 13.2%, PGE_1 10.7%; anti-15-HETE: 8,15- and 5,15-diHETE 1.0%, 12-HETE 0.5%; anti-12-HETE: 15-HETE 0.3%, 5-HETE 0.2%; anti-LTB_4: all < 0.1%; anti-LTC_4: 11-tr-LTD_4 60%, LTD_4 55%, LTD_4-sulfone 10%, LTC_4-sulfone and LTE_4 9%. All tritiated compounds were obtained from the Radiochemical Centre (Amersham, UK).

RESULTS

The Figure shows a separation of lipoxygenase products by HPLC (day 2, not corrected for recoveries). In this chromatogram the absorption at different wave-lengths was monitored (peaks 1–9). Closed dots represent retention times (t_R) of synthetic leukotrienes and mono-HETEs. The detection level was 1 and 5 ng respectively.

After the front two high peaks were measured, indicated by 2 and 3. Immunoassay of these peaks revealed very low activities to the LTC_4 antibody (approx. 0.065 ng max.), as shown in the peaks b and c. These compounds could be tri-HETEs or 11-trans-LT metabolites. As such derivatives have high cross reactivities to the LTC_4 antibody, this is unlikely. Therefore we assume these peaks to be lipoxins (17). Furthermore peaks 4 and d could represent either substances of related structure or 11-trans-LTD_4.

Peak 5 coelutes with LTB_4. The corresponding peak f contains a fraction with a high immunoreactivity to LTB_4 (approx. 2.7 ng max.). In the fractions that were eluted after 20 min, the immunoreactivities to 15- and 12-HETE were determined. Both the absorption of peak 7 and the immunoreactivities represented in peaks g and k respectively suggest that it has the structure of a mono-HETE.

Peak 8 coeluted with 15-HETE and showed immunoreactivity to this compound represented in peak 1. The amount was maximal, approximately 80 ng. There is also cross reactivity with the 12-HETE antibody.

Although no peak in the chromatogram has the t_R of the 12-HETE, two immunoreactive ones were found. These contained small amounts of this substance (< 0.5 ng). The compound representing peak 9 is unknown. Peak j finally indicates the presence of very small amounts of 5-HETE.

Two additional methods were used for the identification of peak 8. The absorption spectrum was measured and shown to be identical to that of synthetic 15-HETE. Secondly, the biological activity of the peak was measured on human small airway smooth muscle (18). It was comparable with the small contractile activity of the standard of 15-HETE and more potent than prostaglandins $F_{2\alpha}$ and E_2.

The calculated amounts determined by radioimmunoassay were of the same order of magnitude as those obtained by measurement of the absorption spectrum at the wavelengths indicated. In the Table the mean concentrations of eicosanoids in mucus obtained from morning stools during 4 days are given. Amounts are corrected for recoveries (15) and expressed in ng/g mucus.

Table Concentrations of eicosanoids in mucus of acute ulcerative proctocolitis obtained from morning stool during 4 days (amounts in ng/g mucus).

6-keto-PGF$_{1\alpha}$	3.7 ± 1.4
TXB_2	9.2 ± 2.5
PGE_2	2.0 ± 0.4
LTB_4	21 ± 3.0
LTC_4	≤0.8
15-HETE	530 ± 65

DISCUSSION

The results indicate that the amounts of LTB_4 and in particular 15-HETE found in the rectal mucus in ulcerative procto colitis are considerably higher than the amounts of PGs. Furthermore smaller amounts are formed of two other mono-HETEs and a di-HETE. Comparatively small, but detectable, amounts of sulphidopeptide LTs were measured by RIA. Two other non treated patients with ulcerative proctocolitis were studied once. Amounts of LTB_4 and 15-HETE were in the same range as those shown is this report. Since it is not possible to obtain mucus from normal individuals other than by biopsies the results reported here only support the possible important role of mono-hydroxy acids in active ulcerative colitis. Measurements of exogenous 15-HETE, LTB_4 and PGE_2 in controls and patients with ulcerative colitis generated from ^{14}C-arachidonic acid labelled biopsies confirmed the ratios found in the herewith presented results (unpublished results).

Recent studies of experimental colitis have established a relationship between LTB_4 and LTC_4 and the severity of the inflammation. Eicosanoid production progressively increased during development of inflammation and infiltration of inflammatory cells (9). In the model used methylprednisolone was not a potent suppressor of LT production (19). In Crohn's disease, enhanced LTB_4, C_4 and D_4 production by colonic mucosa has been described. This formation was dose-dependently inhibited by sulphasalazine and 5-aminosalicylic acid (11). The

sulfidopeptide LTs C_4, D_4 and E_4 are known to increase mucus production (13), vascular permeability (20) and to contract intestinal smooth muscle (21). The chemotactic LTB_4 has also been shown to increase vascular permeability (22), to contract smooth muscle and to increase epithelial secretion (23).

In small bowel mucosa from celiac patients it was found that 15-HETE formation was increased after in vitro challenge with gluten (24). Mono-HETEs have been shown to act as mediators of tracheal mucus secretion (13, 25), and have inflammatory effects in the skin (14). Additionally it was found that 15-HETE inhibits the formation and the chemotactic response of neutrophils to LTB_4, determined in synovial fluid in experimental arthritis (26). This may lead to an increase of cyclooxygenase products. In turn misoprostol, a PGE_1 analogue, can cause an inhibition of the initiating event and the amplification of the inflammation caused by LTB_4 (27).

In our study there was a linear relationship between the 15-HETE and PG contents, whereas the amount of LTB_4 was inversely proportional to that of the PG (data not shown).

The implications of our observation, that 15-HETE is present in high concentrations in mucus, for the treatment of ulcerative colitis, are not clear. Though 5-ASA is a rather weak inhibitor of 5-lipoxygenase the use of more active and specific 5-lipoxygenase inhibitors in ulcerative colitis is being considered (28). Selective 5-lipoxygenase inhibitors, however, could result in increased 15-HETE, diHETE and lipoxin production, and may therefore prove disappointing anti-inflammatory agents for inflammatory bowel disease.

Acknowledgements

The authors are indebted to Drs. J. E. Vincent and M. van Blankenstein for evaluation of the manuscript.

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