Increased leukotriene B_4 release from ileal pouch mucosa in ulcerative colitis compared with familial adenomatous polyposis

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Abstract

Pouchitis may complicate the construction of an ileal pouch after colectomy for ulcerative colitis (UC) but not familial adenomatous polyposis (FAP). To examine whether differences in eicosanoid metabolism might explain why pouchitis is largely confined to UC patients, this study compared arachidonic acid stimulated release of immunoreactive leukotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2) from macroscopically uninflamed pouch mucosal biopsy specimens incubated in vitro from patients with UC and FAP. The study also compared eicosanoid release from inflamed and uninflamed pouches in patients with UC. In uninflamed pouches, median LTB_4 release was nearly twice as high in UC as in FAP ($p=0{\cdot}001$), but there was no significant difference in PGE_2 production. In UC, stimulated eicosanoid release from uninflamed functioning pouch mucosa was not significantly different from that from either ileostomy or defunctioned pouch mucosa. LTB_4 and PGE_2 release were significantly greater from inflamed than uninflamed pouch mucosa in UC ($p=0{\cdot}001$ and $0{\cdot}01$, respectively). Leukotriene synthesis inhibition or receptor antagonism, or both merit therapeutic evaluation in pouchitis. Increased release of LTB_4 from endoscopically normal pouch mucosa suggests increased 5-lipoxygenase activity in patients with UC and could contribute to their predisposition to pouchitis.

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Pouchitis is a syndrome produced by acute inflammation of the mucosa of an ileal reservoir. It is the commonest longterm complication of a restorative proctocolectomy for ulcerative colitis (UC), but is very rare after the same operation for familial adenomatous polyposis (FAP).[1 2] The cause is unknown.

Mucosal overproduction of eicosanoids seems to play a part in the pathogenesis of UC.[3 4] Increased synthesis of the lipoxygenase products of arachidonic acid metabolism, in particular, may contribute to the inflammatory process through their effects on migration and degranulation of neutrophils and on vascular permeability.[3–6] Such changes, once initiated, tend to be self perpetuating through the further recruitment of inflammatory cells into the mucosa, and their activation.

As patients with UC have a particular predilection for developing pouch mucosal inflammation and FAP patients do not, we tested the hypothesis that this might result from differences in the metabolism of eicosanoids. We used a short term in vitro incubation technique to measure the release of leukotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2) from biopsy specimens of uninflamed pouch mucosa, comparing patients with UC with those with FAP.[6 7] To see if changes in eicosanoid release in pouchitis mimic those in rectal mucosa in UC,[3–7] we also assessed eicosanoid release from inflamed pouch mucosa.

Methods

The study was approved by the ethical committee of the City and Hackney Health Authority.

PATIENTS

We studied 28 patients with UC 4–91 months after restorative proctocolectomy, and eight patients with FAP 3–24 months after the operation.

Patients with uninflamed pouches – none of these patients had symptoms, or signs on examination with a paediatric sigmoidoscope, of pouchitis. In the patients with UC, the pouch was in continuity with the faecal stream in 16, of whom six were taking loperamide, while in the remaining six patients it was defunctioned by a proximal loop ileostomy. In all the patients with FAP, the pouch was in continuity with the faecal stream, and two were taking loperamide.

Patients with inflamed pouches – six of the UC patients had one or more episodes (16 episodes in all) of pouchitis diagnosed clinically, endoscopically, and histologically during the study.[1] In all these patients, the pouch was in continuity with the faecal stream. During five episodes, the patient was taking metronidazole, and during two loperamide.

BIOPSY SITES

In each patient studied, three biopsy specimens were taken from adjacent sites in the pouch or on the surface of the loop ileostomy. Two specimens were used to measure the release of LTB_4 and PGE_2, and one was examined histologically.

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IN VITRO INCUBATIONS

A modification of Peskar's technique was used.[6 7] Two specimens (20–40 mg) were separately washed in Tyrode's solution, blot dried, and weighed. Within five minutes, each was placed in a 5 ml glass container for preincubation for 20 minutes in 1 ml continuously gassed (95% O_2/5% carbon dioxide) Tyrode's solution at 37°C. The supernatant was then replaced with 1 ml Tyrode's solution containing arachidonic acid (sodium salt, Sigma, 10 μg/ml) and the specimen incubated for a further 20 minutes. The supernatant was then removed and frozen at −20°C for subsequent assay of its eicosanoid content.

Viability of the incubated specimens was confirmed by their continuing and unaltered release of LTB_4 and PGE_2 with added arachidonic acid during successive 20 minute periods over a total of four hours. In otherwise identical experiments using colorectal mucosal specimens from patients with UC, the coefficient of variation of duplicate adjacent biopsy specimens incubated with arachidonic acid for 20 minutes was 6% (n=6) for LTB_4 release and 8% (n=6) for PGE_2.[7]

ASSAYS

All assays were performed in duplicate on unextracted supernatant within three months of the incubation experiment.

LTB_4 concentration was measured by radioimmunoassay (Amersham International kit no TRK 940). Sensitivity was 0·02 ng/ml, cross reactivity with arachidonic acid <0·05% and with other eicosanoids <0·05%, and the interassay variation 5%. Recovery of LTB_4 added to the supernatant was 79% (72–84) (median (interquartile range)) (n=6). Serial dilution of selected samples showed parallelism with standard solutions. Four random samples were validated by high performance liquid chromatography after extraction and purification of supernatant[8]: the coefficient of variation of the duplicate high performance liquid chromatography and radioimmunoassay measurements was 4%.

PGE_2 was also measured by radioimmunoassay using standard solutions of PGE_2 (Sigma), PGE_2 antisera (gift of Dr J A Salmon) and PGE_2 tracer (Amersham International).[9] The sensitivity of the method was 0·1 ng/ml, and cross reactivity with 6-keto-$PGF_1\alpha$, arachidonic acid, and other eicosanoids was 4·8%, <0·1%, and <1%, respectively. The interassay variation was 6%. Recovery of PGE_2 was 71% (63–80) (n=6), and parallelism of serial dilutions of supernatant with standard solutions was confirmed. Six random samples were validated by rat stomach strip bioassay[10]; the coefficient of variation of the bioassay and radioimmunoassay results was 3%.

Preliminary studies showed that the presence of arachidonic acid in a concentration of 10 μg/ml did not affect the LTB_4 or PGE_2 radioimmunoassays.

The values shown for LTB_4 and PGE_2 release for each patient are the means of the concentrations measured in the supernatants of the two biopsy specimens incubated for each subject.

HISTOLOGICAL ASSESSMENT

The third specimen was used to provide a formalin fixed section stained with haematoxylin and eosin. A pathologist unaware of the group to which the patient belonged provided a semi-quantitative light microscopic score, from 0–3, of the extent of polymorphonuclear leucocyte infiltrate.[11]

STATISTICS

Results are shown as median (interquartile range). Differences among groups were assessed by the Kruskal-Wallis test, and subsequently between groups using Wilcoxon's sum of ranks test (two tailed p values), as appropriate. Correlations were sought using Spearman's rank correlation test.

Results

UNINFLAMED POUCHES IN CONTINUITY WITH THE FAECAL STREAM

The pouch mucosa of patients with UC released significantly more LTB_4 (57 ng/g wet wt/20 min (44–89)) than did the pouch mucosa of patients with FAP (30 (27–35), p=0·001)) (Fig 1). There was a trend towards increased release of PGE_2 in patients with UC (263 ng/g wet wt/20 min (169–446) compared with FAP (158 (123–287), but this did not attain statistical significance (Fig 2).

In UC pouches, eicosanoid release did not correlate with either the score for polymorphonuclear leucocyte infiltration (for LTB_4, $r=-0·16$, p=0·59; for PGE_2, $r=0·08$, p=0·79, or with the time interval since restorative proctocolectomy (LTB_4, $r=-0·37$, p=0·17; PGE_2, $r=-0·28$, p=0·13). There was also no difference in mucosal infiltration of neutrophils in the pouches of patients with

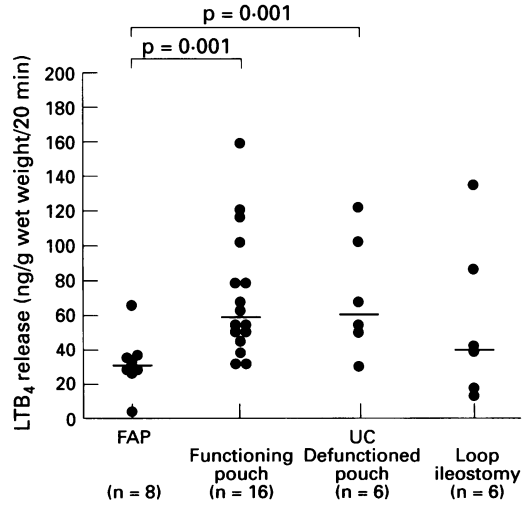

Figure 1: LTB_4 release by mucosal biopsy specimens from endoscopically uninflamed pouches (functioning and defunctioned) and ileostomies in patients with UC and FAP. Horizontal bars denote median values.

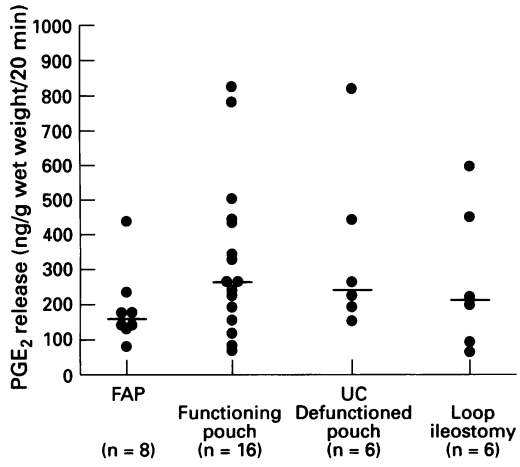

Figure 2: PGE_2 release by mucosal biopsy specimens from endoscopically uninflamed pouches (functioning and defunctioned) and ileostomies in patients with UC and FAP. Horizontal bars denote median values.

UC and FAP (scores 0 (0–1), and 0 (0–1), respectively).

UNINFLAMED DEFUNCTIONED POUCHES AND ILEOSTOMIES

There were no significant differences in release of LTB_4 from defunctioned pouch, ileostomy, and functioning pouch mucosa in patients with UC (Fig 1). There were also no differences in the release of PGE_2 in these categories of specimens (Fig 2). The median release of LTB_4 from defunctioned pouches ($p=0{\cdot}001$), but not ileostomies, in patients with UC was significantly greater than that from pouches of patients with FAP. Although the median release of PGE_2 from defunctioned pouches and from ileostomies in patients with UC was greater than that from the pouches of patients with FAP, the differences were not statistically significant (Figs 1 and 2).

INFLAMED POUCHES IN CONTINUITY WITH THE FAECAL STREAM

Inflamed pouch mucosa released significantly more LTB_4 ($p=0{\cdot}001$) and PGE_2 ($p=0{\cdot}01$) than uninflamed pouch mucosa (Table). Median release of LTB_4, but not PGE_2, from inflamed (and uninflamed) ileal pouch mucosa was also significantly higher than we have previously reported from rectal mucosa in patients with active (and inactive) UC[7] (Table).

Discussion

It is well established that colorectal mucosa in patients with active UC produces more LTB_4 and PGE_2 than does uninflamed mucosa.[3–7] We have shown here that ileal pouch mucosa when inflamed also releases more of these eicosanoids, confirming previous preliminary data.[12] As in UC,[3–7] increased synthesis of leukotrienes probably contributes to pouch mucosal inflammation largely by increasing the recruitment and activation of neutrophils. Excess prostaglandin production may worsen diarrhoea by inducing mucosal secretion of water and electrolytes; conversely, increased prostaglandin synthesis may have a mucoprotective role by increasing mucus release, changing mucosal blood flow, and suppressing immune and inflammatory cell function.[3 4 13] While, in patients with UC, inhibition of prostaglandin synthesis has been shown to be therapeutically ineffective, and indeed possibly harmful,[3 4] limited data suggest that inhibition of mucosal leukotriene synthesis with A-64077 (Zileuton),[14] may have some benefit, at least in patients not already taking aminosalicylates.[15] By analogy, a clinical trial of Zileuton, or, alternatively, an LTB_4 receptor antagonist, would seem appropriate in patients with pouchitis.

It is not known why pouchitis occurs in the ileal mucosa of patients with UC but not those with FAP.[1 2] In this study, we report that patients with UC release more LTB_4 from the mucosa of uninflamed functioning pouches than do patients with FAP.

Polymorphonuclear leucocytes are an important source of LTB_4. In patients with uninflamed pouches, however, there was no difference in the intensity of mucosal infiltration by neutrophils in UC and FAP; furthermore there was no correlation between neutrophil infiltration and LTB_4 release in uninflamed UC pouches. The ability of apparently normal pouch mucosa in patients with UC to release increased amounts of LTB_4 shows that the 5-lipoxygenase enzyme system has been activated. Studies of polymorphonuclear leucocytes in the peripheral blood of patients with UC and Crohn's disease have shown an increased release of LTB_4 compared with healthy volunteers.[16] Our findings may thus reflect activation of 5-lipoxygenase within mucosal neutrophils.

The lack of a significant correlation between the time since restorative proctocolectomy and the mucosal release of LTB_4 suggests that operative trauma, residual 'backwash' ileitis, and drugs used during the perioperative period are unlikely to explain the increase in LTB_4 release by uninflamed pouch mucosa in patients with UC.

Our data are consistent with a predisposition to acute inflammation in the mucosa of

Eiconsanoid release in uninflamed and inflamed pouch mucosa and rectal mucosa in patients with UC. Data for rectal mucosa are taken from Gertner et al.[7] *Results are shown as median (interquartile range) (n)*

	LTB_4 release (ng/g wet wt/20 min)		*PGE_2 release (ng/g wet wt/20 min)*	
	Pouch	*Rectum*	*Pouch*	*Rectum*
Uninflamed	57 (44–89) (16)	30 (24–40) (27)*	263 (169–446) (16)	284 (205–342) (27)
Inflamed	87 (70–94) (16)*	58 (45–73) (16)†§	668 (583–769) (6)†	492 (396–598) (16)§

*p=0·001 from uninflamed pouch mucosa, †=0·01 from inflamed pouch mucosa, ‡=0·01 from uninflamed pouch mucosa, §=<0·05 from uninflamed rectal mucosa.

functioning ileal pouches in patients with UC. They do not, however, show whether this predisposition requires exposure to the faecal stream, as there was no difference between the amounts of LTB_4 released from functioning and defunctioned pouches in patients with UC. Nevertheless, increased LTB_4 synthesis in UC patients with uninflamed pouches suggests a possible role for new drugs inhibiting the synthesis of leukotrienes,[14 15] or antagonising their receptors, for the prevention of pouchitis.

The method used in this study exposed the mucosal biopsy specimens to an excess of arachidonic acid, the substrate for leukotriene production. It is possible that in vivo, without such an excess of substrate the activated 5-lipoxygenase enzyme system would not synthesise excess leukotrienes and would not therefore cause mucosal inflammation. Our failure to detect, in the mucosa of uninflamed pouches, a difference in the infiltrate of polymorphonuclear leucocytes between patients with UC and FAP suggests that excess LTB_4 was not being synthesised in vivo, as it is a powerful chemotactic mediator. It is possible, however, that synthesis of excess LTB_4 (and other leukotrienes), and the subsequent onset of pouchitis, could be triggered by any of a number of hormonal, inflammatory, and immunological factors, which have been shown to promote release of arachidonic acid.[17] Conversely, reducing mucosal arachidonic acid by changing the diet, for example using fish oil,[18 19] may merit evaluation in patients with recurrent pouchitis.

It has been suggested that increased mucosal eicosanoid production in active UC could be explained simply by greater availability of substrate in inflamed than control mucosa.[20] Our results, in which the pouch mucosa of patients with UC released more LTB_4 than the mucosa of FAP patients incubated in a similar excess of arachidonic acid, argue against this proposal, albeit less directly than in analogous studies using colonic mucosa.[7]

Release of LTB_4, but not PGE_2, from both inflamed and uninflamed pouch mucosa exceeded that from rectal mucosa in active and quiescent UC (Table). These comparisons raise the possibility of an inherent difference between LTB_4 release from small and large bowel, for example because of differences in the underlying cell populations, or luminally derived stimuli, responsible for LTB_4 production in the ileum and colon. Previous work, however, in which operative specimens of uninflamed mucosa were stimulated in vitro by calcium ionophore (A23187), showed no difference in eicosanoid release between the ileum and colon.[21] In our study, arachidonic acid was used as the stimulus for LTB_4 release; furthermore, the ileal tissue sampled had been previously transposed to an abnormal site (neorectum or stoma). These factors may explain the discrepancy between the present and previous results[21]: clearly, a study of eicosanoid release from normal ileal and colorectal mucosa obtained colonoscopically is desirable for any further investigation of possible regional differences in leukotriene synthesis in the intestine.

The reason for increased 5-lipoxygenase activity in the uninflamed ileal mucosa of patients with function ileal pouches who have UC, which is thought to be primarily a disease of the colonic mucosa, is not clear. Explaining this may clarify not only the mechanism of pouchitis but also of ulcerative colitis itself.

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