In vivo rectal inflammatory mediator changes with radiotherapy to the pelvis

A T Cole, K Slater, M Sokal, C J Hawkey

Abstract

In vivo changes in the rectal values of eicosanoid inflammatory mediators induced by pelvic radiotherapy were measured to study the pathophysiology of the early radiation bowel reaction. Ten patients having pelvic radiotherapy, aged 57 to 78, had rectal dialysis. Values of the eicosanoids leukotriene B_4 (LTB_4), thromboxane B_2 (TXB_2), and prostaglandin E_2 (PGE_2) were measured before radiotherapy, at the end of radiotherapy, and at least four weeks after radiotherapy. Values of LTB_4 rose with radiotherapy from 0·21 ng.ml^{-1} (median) to 1·14 ng.ml^{-1} (p=0·012); PGE_2 rose from 0·60 ng.ml^{-1} to 1·58 ng.ml^{-1} (p=0·038), and TXB_2 rose from 0·365 ng.ml^{-1} to 1·6 ng.ml^{-1} (p=0·005). The rise in eicosanoid inflammatory mediators may have an important role in the pathophysiology of the early radiation bowel reaction.

(*Gut* 1993; 34: 1210–1214)

The early radiation bowel reaction occurs in radiotherapy to the pelvis in up to 75% of patients treated.[1][2] This normal tissue injury is a limiting factor for radiotherapy dose. The early reaction is associated with symptoms of diarrhoea and nausea[3] and histologically is characterised by an inflammatory cell infiltrate, oedema, ulceration, epithelial cell loss, congestion, and nuclear damage.[4]

The cellular mechanisms underlying this reaction are not clearly understood; they probably entail the triggering of inflammatory reactions by the release of reactive species generated by ionising radiation. Eicosanoid inflammatory mediators include the oxidative products of arachidonic acid prostaglandin E_2 (PGE_2), thromboxane A_2(TXA_2), and leukotriene B_4 (LTB_4). Eicosanoid mediators of inflammation are raised in animal models of radiation injury to the gut.[5]

We have investigated the mechanism of the early radiation bowel reaction in vivo in humans by measuring changes in rectal dialysate values of PGE_2, LTB_4, and TXB_4 (the stable product of TXA_2).

Methods

DESIGN AND AIMS

The study was an open prospective study to investigate the in vivo effect of pelvic radiotherapy on rectal values of the eicosanoid inflammatory mediators PGE_2, TXB_2, and LTB_4 measured in rectal dialysates.

PATIENTS

Ten patients, nine men, one women were studied; all were receiving radical pelvic irradiation for bladder or prostatic cancer. The study was approved by the Nottingham University Hospital Ethics Committee. Patients gave written informed consent. One other patient withdrew from the trial because of development of a painful rectum during radiotherapy. The study was conducted in a subregional radiotherapy centre and an academic department of gastroenterology and therapeutics.

RADIOTHERAPY

Radiotherapy was given as clinically indicated using either a 6 MV or 8 MV linear accelerator. The dose to the rectum was calculated from the planning computed tonography. The maximum and minimum radiation dose to the rectum was calculated in the plane of the planning slice for each patient. The dialysis technique sampled this area. Radiotherapy for cancer of the bladder was typically given in two phases; phase I tumour dose of 40 Gray in 20 daily fractions over four weeks and phase II 20 Gray in 10 daily fractions over two weeks, the different phases being distinguished by the fields used but always irradiating a similar volume of the rectum. Radiotherapy for cancer of the prostate was also in two phases but with a 25 Gray phase two, in 15 fractions.

CLINICAL SCORING OF ABDOMINAL SYMPTOMS

A clinical score was derived at the time of each dialysis by adding scores for: abdominal pain (none=0, mild=1, moderate=2, severe=3); the stool frequency (1 or less per day=0, 2 to 3=1, 4 to 5=2, 6 to 9=3, 10 or more=4); the visible presence of blood in the stool (none=0, a trace=1, more than a trace=2); and the stool consistency (normal=0, semiformed=1, liquid=2). All medicines taken by patients were recorded.

RECTAL DIALYSIS

Patients had rectal dialysis at baseline and then at the end of the course of radiotherapy. Six patients had a third dialysis after at least a further four weeks. Rectal dialysis bags were made from ⅜ inch Visking Tubing (Medicell) with a molecular weight cut off of 12 000–14 000 filled with Rheomacrodex (Pharmacia) as previously described.[6] Patients evacuated their bowels before rectal dialysis. Dialysis bags were inserted per rectum for two hours and then removed. A two hour dialysis time does not achieve equilibrium values of the eicosanoid measured but reflects epithelial values and has been used by other authors.[7] We did not discard stained dialysates. The treatment time was chosen

Department of
Therapeutics, University
Hospital, Nottingham
A T Cole
C J Hawkey

Department of Clinical
Oncology, General
Hospital, Nottingham
K Slater
M Sokal

Correspondence to:
Professor C J Hawkey,
Department of Therapeutics,
University Hospital,
Nottingham NG7 2UH.
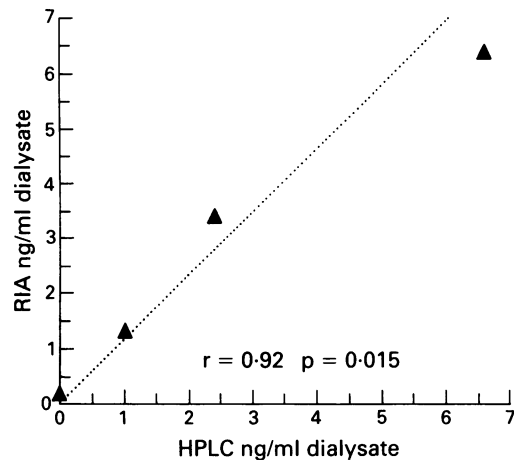
Accepted for publication
19 January 1993

Figure 1: Correlation of radioimmunoassay and high performance liquid chromatography (HPLC) measurements of spiked dialysate LTB₄.
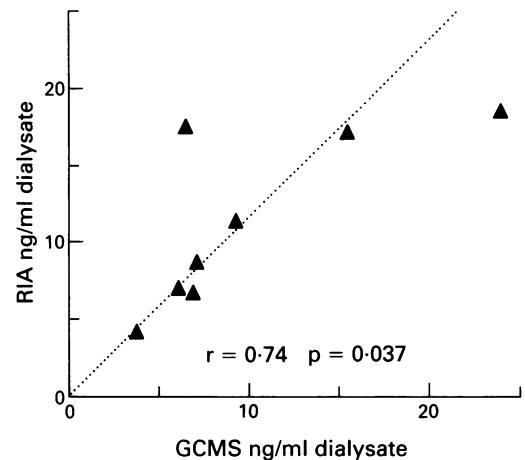

Figure 3: Correlation of radioimmunoassay and gas chromatography and mass spectrometry (GCMS) measurements of dialysate TXB₂.

because patients are able to tolerate it well and variable early expulsion is avoided. Dialysis was performed at the same time of day for each subject. Unextracted samples were stored at −40°C and samples extracted into methanol were stored at −20°C. Samples from individual subjects were assayed together.

RADIOIMMUNOASSAY OF EICOSANOIDS

Dialysates were assayed by radioimmunoassay. The assays were validated against high performance liquid chromatography (HPLC) for the LTB₄ assay and against gas chromatography and mass spectrometry (GCMS)[8] for the TXB₂ and PGE₂ assay.

LTB₄

Samples were extracted for LTB₄ assay. One ml of rectal dialysate sample was mixed with 1 ml of HPLC grade methanol (Fisons). This precipitated out the dextran which was spun down at 3500 rpm at 4°C for 10 minutes. Samples of each supernatant (1·67 ml) were taken and acidified to pH 4 with 0·5% trifluoroacetic acid (Sigma) using an autotitrator (Radiometer, Copenhagen). Fifty μl of ³H LTB₄ (approximately

1000 cpm, Amersham International) was added as a recovery standard and the sample further diluted with 2 ml of water at Ph 3·5. Each sample was loaded onto a C8 reverse phase silica cartridge with metal frits (Bond-elute, Anachem) previously prepared with washes of methanol and water at pH 3·5; washed with 1 ml each of HPLC grade water, 10% ethanol and hexane, and finally eluted with 1 ml of methanol.[9] Extracted samples were stored in sealed polypropylene tubes at −20°C and subsequently assayed with a commercial radioimmunoassay (Amersham International). The assay has a cross reactivity for 20-OH LTB₄ of 3·9%, with lower cross reactivity to other eicosanoids. Samples were measured in comparison with extracted standards prepared in duplicate.

PGE₂ AND TXB₂

PGE₂ and TXB₂ were assayed in diluted unextracted dialysates by radioimmunoassay as previously described.[10] The PGE₂ antibody (Sigma) has a cross reactivity of 51% to PGE₁ but not to other eicosanoids. TXB₂ antibody was obtained from Levine.[11] The antibody has a cross reactivity of <1% to PGE₂ and other eicosanoids.[11]
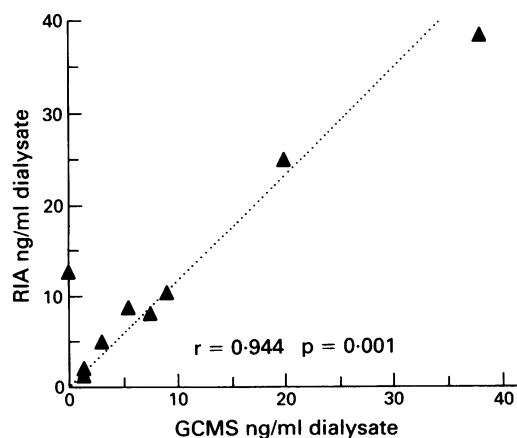

Figure 2: Correlation of radioimmunoassay and gas chromatography and mass spectrometry (GCMS) measurements of dialysate PGE₂.

VALIDATION OF RADIOIMMUNOASSAYS

Healthy volunteer dialysate spiked with 1–5 ng.ml LTB₄ was assayed by both HPLC and radioimmunoassay. Results correlated well (r= 0·92, p=0·015) (Fig 1). Dialysates from patients with ulcerative colitis were assayed both by GCMS and by radioimmunoassays. For PGE₂ (Fig 2) and TXB₂ (Fig 3), there was a good correlation for both radioimmunoassays: PGE₂ (r=0·944, p=0·001) and TXB₂ (r=0·736, p= 0·037). In spiking experiments 10 ng.ml⁻¹ of LTB₄, PGE₂, and TXB₂ were added to n=6 samples. Separate unspiked aliquots of the same dialysate were also assayed. The measured differences were not significantly different from the known addition: LTB₄ 88% recovery (confidence intervals, 73–103%); TXB₂ 108% recovery (82–134%); PGE₂ recovery 112% (91–113%).

TABLE *Details of patients*

Age	Diagnostic group	Radiotherapy		Clinical score		Opiate and anti-diarrhoeal medicines	
		Max	Min	Before	After	Before	After
78	B	32·2	19·8	0	0		
62	B	46·8	31·2	0	0		Loperamide
57	P	64·3	50·3	2	2		
78	B	50·5	18·0	1	5		Codeine
74	B	60·4	21·8	2	2		Coproxamol
64	B	59·8	23·0	0	1		Dihydrocodeine
74	B	51·6	32·0	0	2		
75	B	60·0	21·4	2	3		
66	P	45·2	24·4	4	5		Coproxamol Loperamide
74	B	61·2	36·2	0	4	Coproxamol	

B=bladder cancer, P=prostate cancer. Radiotherapy=calculated total dose in planning slice in Gy (max=maximum dose, min=minimum dose). Before=baseline before treatment; After=at end of radiation treatment.

SENSITIVITY
Sensitivity was assessed as the threshold detection at 90% B/Bo for the individual radioimmunoassays. The LTB_4 assay threshold was 0·2 ng.ml^{-1} dialysate at the strongest dilution used for assay (1 in 4). The TXB_2 assay threshold was 0·1 ng.ml^{-1} and the PGE_2 assay threshold was 0·6 ng.ml^{-1}.

REPRODUCIBILITY OF ASSAYS
Paired specimens were measured in the same assay. The intra assay variability of LTB_4 at 2·46 ng.ml^{-1} showed a standard deviation of 0·5 ng.ml^{-1}, coefficient of variation 20·13%. PGE_2 assay showed an intra assay variability at 8·8 ng.ml^{-1} of standard deviation 0·92 ng.ml^{-1}, coefficient of variation 11·5%. TXB_2 assay had an intra assay variability at mean 6·55 ng.ml^{-1} of
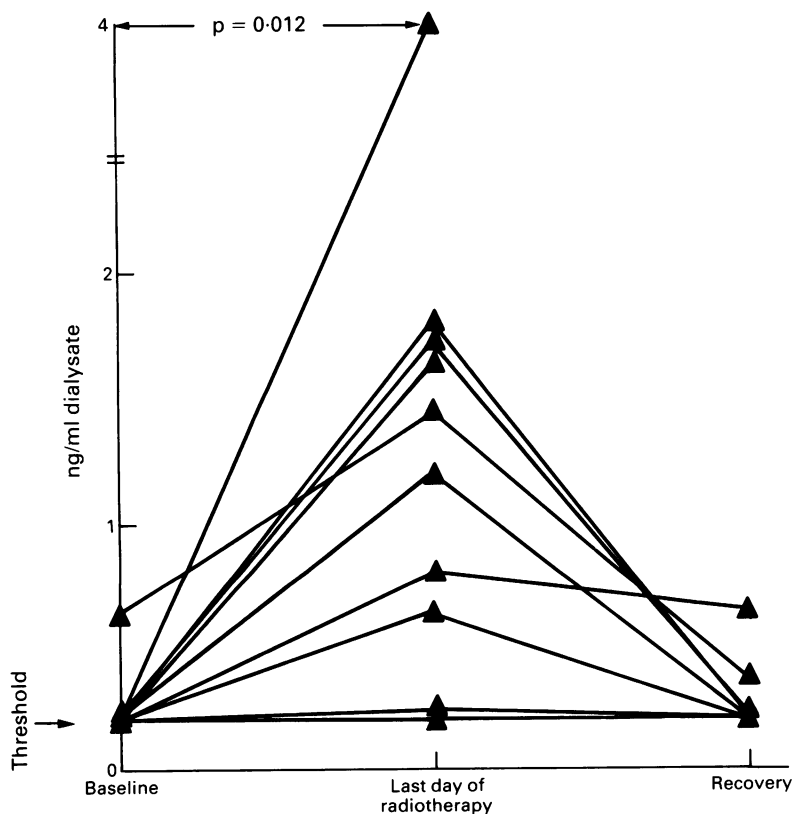
0·26 ng.ml^{-1} standard deviation, coefficient of variation 4·01%.

FAECAL COLORATION OF DIALYSATES
Coloration of the dialysates was assessed as light absorbency at 488 nm measured by spectrophotometry (Pye Unicam) (stained dialysates typically have a broad peak at this frequency).

STATISTICAL ANALYSIS
The Wilcoxon signed rank test for matched pairs was used for statistical analysis.

Results

RADIATION DOSE TO THE RECTUM
The Table shows the dose ranges for the individual patients. The minimum dose given to the rectum in the plane of the radiotherapy planning slice ranged from 18 to 50·3 Gy and the maximum dose ranged from 32·2 to 64·3 Gy.

CHANGES IN CLINICAL SCORES AND DRUG USAGE
The Table shows the drugs given and clinical scoring. Six of the patients used an opiate or an anti-diarrhoeal preparation during the radiotherapy. Clinical scoring rose by 1 (median, 0 to 4) points. There tended to be a correlation between the clinical score at the end of radiotherapy and the values of PGE_2 (r=0·49, p=0·076 Spearman), TXB_2 (r=0·47, p=0·085), and LTB_4 (r=0·37, p=0·15).

CHANGES IN DIALYSATE COLORATION
The colour of the dialysate was not significantly different between the three dialysis periods changing from an absorbency of 0·56 (median, range 0·2 to 4·56) at baseline to 1·1 (0·36 to 7·52, difference p=0·37 Wilcoxon) after radiotherapy and to 1·09 (0·08 to 5·06, difference from post radiotherapy dialysis p=0·12) four weeks after radiotherapy.

CHANGES IN INFLAMMATORY MEDIATORS AFTER RADIOTHERAPY
Radiotherapy caused rectal eicosanoid value to rise. The finish of radiotherapy caused values to fall back towards baseline after four weeks. Figure 4 shows the results for LTB_4. There is a consistent and significant rise in the LTB_4 from 0·21 (median, range 0·20 to 0·64) before radiotherapy to 1·14 (0·20 to 4·0) after radiotherapy (median rise 0·89 ng.ml^{-1}, p=0·012). In those subjects studied again at four to eight weeks after radiotherapy LTB_4 values fell by 1 ng.ml^{-1} (0 to −1·6, p=0·028).

TXB_2 rose after radiotherapy from 0·36 ng.ml^{-1} (0·10 to 0·88) before radiotherapy to 1·6 (0·26 to 3·28, (median rise 1·25 ng.ml^{-1} (p= 0·005)) and then fell four weeks after radiotherapy stopped by 1·33 ng.ml^{-1} (+0·05 to −3·01, p=0·028) (Fig 5). PGE_2 rose after radiotherapy from 0·6 ng.ml^{-1} (0·60 to 2·4) to 1·58

Figure 4: Effect of radiotherapy on values of LTB_4 in rectal dialysis.

$ng.ml^{-1}$ (median 0·60 to 9·6) with a median rise of 0·44 $ng.ml^{-1}$, p=0·038) and then fell by −0·48 $ng.ml^{-1}$ (median, range +0·92 to −1·56, p=0·173) (Fig 6).
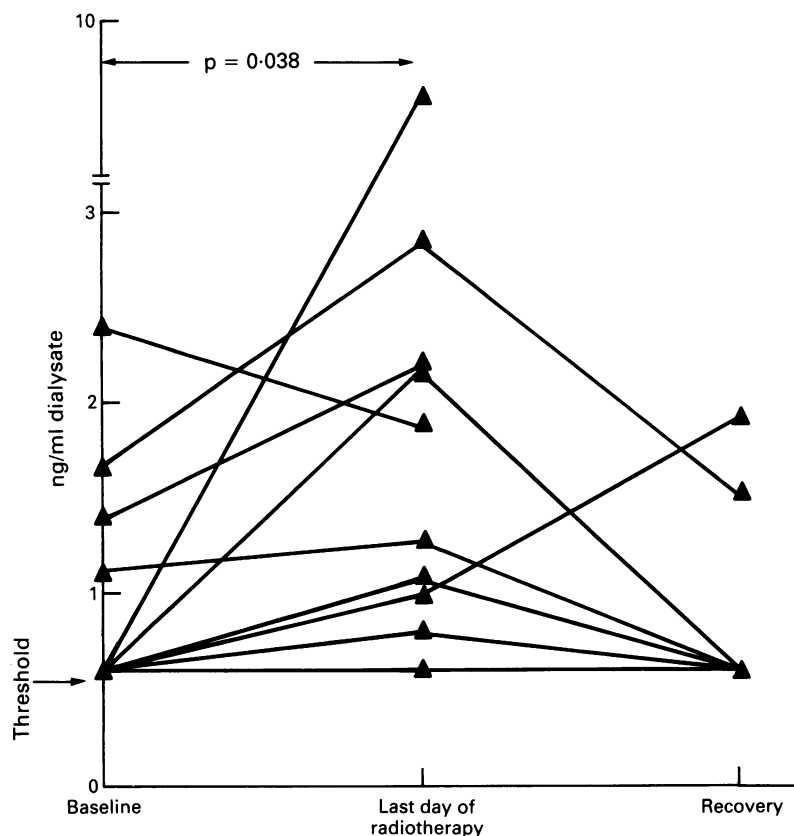

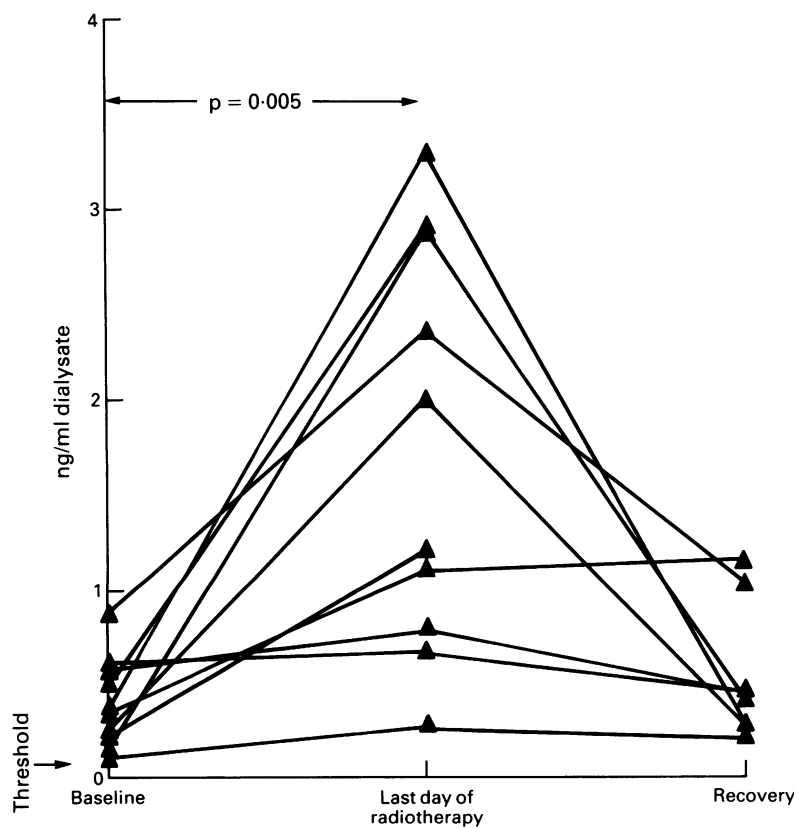Figure 5: Effect of radiotherapy on values of PGE_2 in rectal dialysis.

Figure 6: Effects of radiotherapy on values of TXB_2 in rectal dialysis.

Discussion

We have shown that rectal dialysate values of LTB_4, TXB_2, and PGE_2 rise after pelvic radiotherapy. These mediators are probably responsible for pathological changes because it is known that these mediators can induce an inflammatory response by changing capillary permeability, inducing inflammatory cell infiltration, and activating the inflammatory cells. Leukotriene B_4 causes neutrophil chemotaxis, adherence, aggregation, degranulation, and an oxidative burst.[12] Thromboxane causes adherence of neutrophils and vasoconstriction.[13][14] Prostaglandin E_2 promotes oedema formation and chloride secretion[15] (however, it also has some anti-inflammatory action such as inhibiting neutrophil degranulation). The changes in mediators tended to correlate with the clinical score. This also supports the proposed pathogenical role. We did not look at the histological correlate of the inflammatory mediators as for ethical reasons we did not perform sigmoidoscopy and rectal biopsy.

The mechanism of induction of eicosanoids may be either primary or secondary. A trigger for leukotriene synthesis may be the formation by ionising radiation of lipid peroxides, which may in turn promote synthesis of leukotrienes by LTA_4 hydrolase.[16] Evidence supportive of this mechanism is that vitamin E, a fat soluble free radical scavenger, reduces the formation of fluid in a rat model of radiation enteritis.[17]

Other biological effects of radiation may also be important. Radiation induces nuclear regulating proteins, these are able to regulate cytokines,[18] which may themselves have secondary effects on eicosanoids. Radiation damage to the epithelium[19][20] will lead to a breakdown of the mucosal barrier to chemoattractants such as FMLP,[21] bacteria, and toxins, resulting in a secondary inflammatory response. Radiation may induce diarrhoea by a non- inflammatory mechanism such as bile acid malabsorption[22] (although only as a feature of late radiation damage to the small bowel).

Although we did not study any patients with severe symptoms, our findings seem to be clinically relevant. Clinical scores rose in 6/10 patients, anti-diarrhoeal drugs were used in 3/10, and opiate containing preparation in a further three. The changes in eicosanoids reflected the clinical symptoms; and we would expect them to be greater in severely symptomatic patients. We do not know whether our findings of early changes in rectal dialysate eicosanoid values relate to the risk of late complications.

In pelvic radiotherapy the early rise in rectal leukotrienes, thromboxane, and prostaglandins has a potential treatment implication as inhibitors are available. Aspirin will inhibit both thromboxane and prostaglandin synthesis; it has been shown to ameliorate the early radiation bowel reaction.[23] Steroids inhibit the synthesis of all the eicosanoids; they reduce late radiation induced fibrosis in experimental models.[24] Prostaglandins, however, may be radioprotective.[17][20] Therefore, selective inhibitors that inhibit leukotrienes and thromboxanes but not prostaglandins may be of value. Drugs such as 5-lipoxygenase inhibitors[25] and TXB_2 synthase

inhibitors and receptor antagonists[26] are becoming available. Specific prophylactic inhibition of eicosanoid inflammatory mediators should be investigated as a way of limiting normal tissue injury to the rectum during pelvic radiotherapy.

1 Galland RB, Spence J, eds. *Radiation enteritis.* London: Edward Arnold, 1990.
2 Gilinsky NH, Burns DG, Barbezat GO, Levin W, Myers HS, Marks IN. The Natural History of Radiation-inducted Proctosigmoiditis. An Analysis of 88 Patients. *Q J Med* 1983; ??: 40–53.
3 Bosch A, Frias Z. Complications after radiation therapy for cervical carcinoma. *Acta Radiol Oncol Radiat Phys Biol* 1977; **16**: 53–62.
4 Weisbrot IM, Liber AF, Gordon BS. The effects of therapeutic irradiation on colonic mucosa. *Cancer* 1975; **36**: 931–40.
5 Harding RK, Leach KE, Prud'home-Lalonde L, Ferrarotto CL. Release of inflammatory mediators from irradiated gastrointestinal tissue. *Gastroenterology* 1990; **102** (suppl): A258.
6 Lauritsen K, Laursen LS, Bukhave K, Rask-Madsen J. Effects of topical 5-aminosalicylic acid and prednisolone on prostaglandin E₂ and leukotriene B₄ levels determined by equilibrium in vivo dialysis of rectum in relapsing ulcerative colitis. *Gastroenterology* 1986; **91**: 837–44.
7 Rampton DS, Sladen GE, Youlten LJ. Rectal mucosal prostaglandin E₂ release and its relation to disease activity, electrical potential difference, and treatment in ulcerative colitis. *Gut* 1980; **21**: 591–6.
8 Barrow SE, Ward PS, Sleightholm MA, Ritter JM, Dollery CT. Cigarette smoking: profiles of thromboxane and prostacyclin derived products in human urine. *Biochim Biophsys Acta* 1989; **993**: 121–7.
9 Powell WS. Rapid extraction of oxygenated metabolites of arachidonic acid from biological samples using octadecylsilyl silica. *Prostaglandins* 1980; **20**: 947–57.
10 Hawkey CJ, Trulove SC. Effect of prednisolone on prostaglandin synthesis by rectal mucosa in ulcerative colitis: an investigation by laminar flow bioassay and radioimmunoassay. *Gut* 1981; **22**: 190–3.

11 Alam I, Ohuchi K, Levine L. Determination of cyclooxygenase products and prostaglandin metabolites using high-pressure liquid chromatography and radioimmunoassay. *Anal Biochem* 1979; **93**: 339–45.
12 Palmblad J, Malmsten CL, Udén AM, Rådmark ?, Engsted L, Samuelsson B. Leukotriene B₄ is a potent and stereospecific stimulator of neutrophil chemotaxis and adherence. *Blood* 1981; **58**: 658–61.
13 Gresele P, Deckmyn H, Nenci GG, Vermylen J. Thromboxane synthase inhibitors, thromboxane receptor antagonists and dual blockers in thrombotic disorders. *Trends Pharmacol Sci* 1991; **12**: 158–63.
14 Goldman G, Welbourn R, Valeri CR, Shepro D, Hechtman HB. Thromboxane A₂ induces leukotriene B₄ synthesis that in turn mediates neutrophil diapedesis via CD 18 activation. *Microvasc Res* 1991; **41**: 367–75.
15 Robert A. Prostaglandins and the gastrointestinal tract. In: Johnson LR, ed. *Physiology of the gastrointestinal tract.* New York: Raven Press, 1991: 1407–34.
16 Haeggstrom JZ, Wetterholm A, Samuelsson B. Leukotriene A₄ hydrolase: a zinc metalloenzyme with dual enzymatic activities. In: Nigam S, Honn KV, Marnett LJ, Walden TL Jr, eds. *Eicosanoids and other bioactive lipids in cancer inflammation and radiation injury.* Boston: Kluwer Academic, 1992: 39–42.
17 Empey LR, Papp JD, Jewell LDA, Fedorak RN. Mucosal protective effects of vitamin E and misoprostol during acute radiation induced enteritis in rats. *Dig Dis Sci* 1992; **37**: 205–14.
18 Brach MA, Hass R, Sherman ML, Gunji H, Weichselbaum R, Kufe D. Ionizing radiation induces expression and binding activity of the nuclear ractor κβ. *J Clin Invest* 1991; **88**: 691–5.
19 Bloomer WD, Hellman S. Normal tissue response to radiation therapy. *N Engl J Med* 1975; **293**: 80–3.
20 Hanson WR, DeLaurientis K. Comparison of in vivo murine intestinal radioprotection by E-prostaglandins. *Prostaglandins* 1987; **33**: 93–104.
21 Chadwick VS, Schlup MMT, Cooper BT, Broom MF. Enzymes degrading bacterial chemotactic F-met peptides in human ileal and colonic mucosa. *J Gastroenterol Hepatol* 1990; **5**: 375–81.
22 Arlow FL, Dekovich AA, Priest RJ, Beher WT. Bile acids in radiation-induced diarrhoea. *South Med J* 1987; **80**: 1259–61.
23 Mennie AT, Dalley V. Aspirin in radiation induced diarrhoea. *Lancet* 1973; i: 1131.
24 Evans ML, Graham MM, Mahler PA, Rasey JS. Use of steroids to suppress vascular response to radiation. *Int J Radiat Oncol Biol Phys* 1987; **13**: 563–7.
25 McMillan RM, Walker ERH. Designing therapeutically effective 5-lipoxygenase inhibitors. *Trends Pharmacol Sci* 1992; **13**: 323–30.
26 Fiddler GT, Lumley P. Preliminary clinical studies with thromboxane synthase inhibitors and thromboxane receptor blockers. *Circulation* 1990; **81** (suppl): 169–78.