

Increased arachidonic acid composition of phospholipids in colonic mucosa from patients with active ulcerative colitis

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SUMMARY The long chain fatty acid composition of phospholipids in colonic mucosa was determined by high performance liquid chromatography in nine patients with active ulcerative colitis and eight healthy controls. The arachidonic acid composition was 12.5 ± 1.4 mol % (mean \pm 2 SEM) in the inflamed colonic mucosa from the patients with active ulcerative colitis and 6.8 ± 1.2 mol % in the intact mucosa from healthy controls ($p < 0.001$). In the inflamed colonic mucosa, oleic acid and palmitoleic acid were concomitantly decreased ($p < 0.001$ and $p < 0.02$, respectively), while docosahexaenoic acid was increased ($p < 0.05$). Histopathological examination showed that there was a three fold increase in the cell density of inflammatory infiltrate in the lamina propria of the inflamed colonic mucosa ($p < 0.001$). The cell density of inflammatory infiltrate correlated with the arachidonic acid composition of phospholipids in colonic mucosa ($r = 0.89$, $p < 0.005$). These findings indicate that inflammation alters the long chain fatty acid composition of phospholipids in colonic mucosa. The observed increase in the arachidonic acid composition of phospholipids in inflamed colonic mucosa may contribute to the enhanced arachidonic acid metabolism in patients with active ulcerative colitis.

Arachidonic acid, incorporated into the two position of phospholipids,¹ is an integral component of cell membranes,² and its metabolites formed *via* both the cyclooxygenase and lipoxygenase pathways are thought to be an important mediator of inflammation in ulcerative colitis.³ Raised concentrations of prostaglandins are found in inflamed colonic mucosa, serum, urine, and stool of patients with active ulcerative colitis.⁴⁻⁶ Sharon and Stenson⁷ have recently reported that rectal biopsy specimens from patients with active ulcerative colitis contain large amounts of lipoxygenase products, 5-hydroxyeicosatetra-enoic acid (5-HETE) and leucotriene B₄, potent chemotactic agents recruiting neutrophils into areas of inflammation.⁸⁻¹² A likely explanation

for these observations is related to the enhanced activation of phospholipases which are responsible for controlling free arachidonic acid levels, as tissue levels of active kallikrein which releases kinin and therefore activates phospholipases are increased in patients with active ulcerative colitis.¹³ There is little information, however, on esterified fatty acids stored as phospholipids in colonic mucosa in patients with active ulcerative colitis. The present study was undertaken to elucidate the influence of inflammation on the long chain fatty acid composition of phospholipids in colonic mucosa in these patients.

On the other hand, the histologic features of ulcerative colitis in an active phase are the presence of mucosal inflammation with an increase in the lymphocyte and plasma cell content of the lamina propria and focal polymorphonuclear leucocyte infiltration, sometimes forming crypt abscesses.¹⁴ These inflammatory cells are known to contain large amounts of esterified fatty acids.^{15,16} Therefore, the

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cell density of inflammatory infiltrate in the lamina propria was also determined histologically.

Methods

PATIENTS

Studies were carried out on nine patients with active ulcerative colitis (six men and three women, age range 18–54 years) and eight healthy controls (five men and three women, age range 21–67 years), after receiving informed consent. The diagnosis of the disease was based on the clinical picture, laboratory values, sigmoidoscopy, radiographic examination using air barium double contrast technique, and histologic characteristics of biopsy sample. Of the nine patients with active ulcerative colitis, six had total involvement of the large colon, and one had left sided involvement. The remaining two had the disease confined to the rectum and sigmoid colon. Patients with previous surgical treatment were excluded from the present study. All patients were instructed to take 3–4 g oral sulphasalazine per day. Of the nine patients, five received 20–40 mg of oral prednisolone per day.

In all patients, two biopsy specimens were taken endoscopically from the mucosa of the sigmoid colon, 15–30 cm from the anus without complication. The distance between these two biopsy sites was negligible. All the patients with active ulcerative colitis had inflamed colonic mucosa, which was extremely reddened and friable and bled spontaneously with discrete ulcers and erosions. On the other hand, healthy controls had intact colonic mucosa without any signs of inflammation. Lipids were extracted from one biopsy specimen. After the separation of phospholipids from the other lipids by thin layer chromatography, the long chain fatty acid composition of phospholipids was determined by high performance liquid chromatography (HPLC). The other biopsy specimen was used for histopathological examination to quantify the cell density of inflammatory infiltrate in the lamina propria of the colonic mucosa.

ANALYSIS OF LONG CHAIN FATTY ACID COMPOSITION OF PHOSPHOLIPIDS IN COLONIC MUCOSA

The one biopsy specimen was put into a test tube containing 3 ml chloroform-methanol (2:1, v/v), and homogenised with a Polytron homogeniser (Kinematica Co, Switzerland) for 120 s, and lipids were extracted from the tissue. Phospholipids were separated from the other lipids by TLC in one dimension on 5×20 cm×0.25 mm silica plate (Silica-Gel 60 F₂₅₄, E Merck Co, Darmstadt, FRG), which was developed with petroleum ether-diethyl ether-acetic

acid (70:30:1, v/v). Phospholipid fraction was scraped off from the TLC plate, and extracted with 6 ml methanol containing 30 nmol margaric acid (internal standard). The solvent was removed with N₂ stream, and the residue was saponified with 1 ml of 1 M KOH-ethanol (2:8, v/v) at 90°C for 10 minutes. After acidification with 6 N HCl, the free fatty acids were extracted with 5 ml of diethyl ether and followed by evaporation under N₂ stream. The residual fatty acids were dissolved in 50 µl of ethanol and converted to their 2-nitrophenylhydrazine (2-NPH) derivatives as previously described.¹⁷ Briefly, 100 µl 0.02 M 2-NPH hydrochloride in ethanol, 100 µl 0.25 M 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride in H₂O, and 100 µl 3% (v/v) pyridine in ethanol were added, and the mixture was heated at 60°C for 20 minutes. After the addition of 50 µl 15% (w/v) KOH in methanol-H₂O (4:1, v/v), the mixture was further heated at 60°C for 15 minutes. To the resultant hydrazide mixture, 2 ml of 0.03 M phosphate buffer (pH 6.4)–0.5 N HCl (3.8:0.4, v/v) were added. The fatty acid hydrazides were extracted with 1.5 ml n-hexane, and the solvent was evaporated under N₂ stream. The residue was dissolved in 100 µl of methanol and was then submitted to HPLC measurements.

A Shimadzu LC-5A liquid chromatograph (Shimadzu Seisakusho Co, Kyoto, Japan) equipped with a Shimadzu SPD-2A variable-wavelength UV detector was used. The UV detector was set to monitor the absorbance at 230 nm. A C₈ reversed-phase column, 250 mm×4.6 mm ID, packed with YMC-C8 (5 µm in particle size) (Yamamura Chemical Institute, Kyoto, Japan) was maintained at 30°C and eluted isocratically with acetonitrile-H₂O (85:15, v/v). The pH of the solvent was maintained at 4.5, and the flow rate was 1.2 ml/min.

QUANTIFICATION OF CELL DENSITY OF INFLAMMATORY INFILTRATE IN LAMINA PROPRIA OF COLONIC MUCOSA

The other biopsy specimen was fixed with 10% formalin, embedded in paraffin, and cut into section 2 µm in thickness. After staining with haematoxylin and eosin (H&E), the material was viewed independently by one author (AS) who was unaware of the results of HPLC measurements, using a Olympus BHS 324 light microscope (Olympus Co, Tokyo, Japan) equipped with a Olympus PM 10 ADS-1 photomicrographic system. In each specimen, 10 photomicrographs of 250× magnification were randomly taken using 24×36 mm colour film, and total inflammatory cells present in the lamina propria were counted so that the cell density of inflammatory infiltrate in the lamina propria could be quantified.

STATISTICAL ANALYSIS

The data obtained were expressed using the 95% confidence intervals (mean \pm 2SEM) and the range of observations. Comparisons of data between patients and healthy controls were calculated by the non-paired Student's *t* test. Probability values <0.05 were considered statistically significant.

Results

ANALYSIS OF LONG CHAIN FATTY ACID COMPOSITION OF PHOSPHOLIPIDS IN COLONIC MUCOSA

The mean value for the arachidonic acid (C_{20:4}) composition of phospholipids in colonic mucosa was 12.5 \pm 1.4 mol % (range 9.8–15.0) in patients with active ulcerative colitis, being significantly (*p*<0.001) higher than the 6.8 \pm 1.2 mol % (range 3.5–8.8) in healthy controls. In the inflamed colonic mucosa from patients with active ulcerative colitis, both oleic acid (C_{18:1}) and palmitoleic acid (C_{16:1}) were decreased (*p*<0.001 and *p*<0.02, respectively), whereas docosahexaenoic acid (C_{22:6}) was increased (*p*<0.05). The proportions of eicosapentaenoic acid (C_{20:5}) and dihomo-*r*-linolenic acid (C_{20:3}) were considerably lower than that of arachidonic acid, and no significant difference was observed between the inflamed and intact colonic mucosa. There was no significant difference in the proportions of individual saturated fatty acids between the two groups. Similarly, the ratio of unsaturated fatty acids to saturated fatty acids did not differ significantly between the two groups (Table). Figure 1 represents HPLC profiles of long chain fatty acids of phospholipids in inflamed colonic mucosa and normal colonic mucosa.

CELL DENSITY OF INFLAMMATORY INFILTRATE IN LAMINA PROPRIA OF COLONIC MUCOSA

In an area of 10 photomicrographs (0.14 mm²), the total number of inflammatory cells present in the lamina propria was 1276.7 \pm 202.2 cells (range 801–1821) in the inflamed colonic mucosa from patients with active ulcerative colitis and 375.9 \pm 52.0 cells (range 212–437) in the intact colonic mucosa from healthy controls (*p*<0.001). The average cell density of inflammatory infiltrate for the inflamed colonic mucosa was 9119.0 \pm 1444.1 cells/mm² (range 5721–13 007), which was significantly (*p*<0.001) higher than the 2684.8 \pm 370.8 cells/mm² (range 1514–3121) for the intact mucosa from healthy controls. The cell density of inflammatory infiltrate in the lamina propria correlated with the arachidonic acid composition of phospholipids in colonic mucosa (*r*=0.89, *p*<0.005) (Fig 2). In the inflamed colonic mucosa, the inflammatory infiltrate consisted predominantly of lymphocytes and plasma cells.

Discussion

The results of the present investigation show that the arachidonic acid composition of phospholipids in colonic mucosa is increased in patients with active ulcerative colitis. This observation indicates that inflammation alters the composition of fatty acids stored as phospholipids in colonic mucosa. The increase in the proportion of esterified arachidonic acid may have direct relevance to the enhanced arachidonic acid metabolism in such patients, as phospholipases catalyse the hydrolysis of esterified arachidonic acid to cause release of free arachidonic acid, which is the most important source of both the cyclooxygenase and lipoxygenase products. Zeitlin

Table Composition of long chain fatty acids of phospholipids in inflamed colonic mucosa obtained from patients with active ulcerative colitis (*n*=9) and in normal colonic mucosa from healthy controls (*n*=8). Data are expressed as mean \pm 2 SEM and the range of observations.

Fatty acid composition of phospholipids (mol %)	Inflamed colonic mucosa (<i>n</i> =9)		Normal colonic mucosa (<i>n</i> =8)	
	Mean \pm 2 SEM	Range	Mean \pm 2 SEM	Range
C _{12:0}	0.9 \pm 0.4	0.3–1.5	0.7 \pm 0.4	0.1–1.5
C _{14:0}	2.3 \pm 1.2	1.3–6.8	1.8 \pm 0.6	0.5–2.8
C _{14:1}	0.1 \pm 0.1	tr.–0.2	0.1 \pm 0.1	tr.–0.2
C _{16:0}	25.5 \pm 1.2	21.7–26.6	24.7 \pm 4.6	19.0–32.3
C _{16:1}	1.3 \pm 0.2*	0.8–1.6	2.3 \pm 0.8	1.0–4.5
C _{18:0}	17.0 \pm 1.0	16.0–20.7	14.8 \pm 2.8	8.3–22.2
C _{18:1}	18.5 \pm 1.2†	15.9–22.2	26.8 \pm 2.2	23.8–31.7
C _{18:2}	15.0 \pm 1.0	12.9–17.2	16.1 \pm 2.2	9.2–18.4
C _{18:3}	0.3 \pm 0.2	tr.–0.8	0.4 \pm 0.2	0.1–1.0
C _{20:3}	0.1 \pm 0.2	tr.–0.5	0.1 \pm 0.2	tr.–0.5
C _{20:4}	12.5 \pm 1.4†	9.8–15.0	6.8 \pm 1.2	3.5–8.8
C _{20:5}	1.9 \pm 1.0	0.7–5.5	2.3 \pm 0.8	0.5–3.6
C _{22:6}	4.6 \pm 0.6‡	3.3–5.8	3.1 \pm 1.0	1.3–4.9
Unsaturated fatty acids	54.3 \pm 2.4	47.7–58.2	58.0 \pm 6.0	42.4–65.9

**p*<0.02; †*p*<0.001; ‡significantly different from corresponding control value at *p*<0.05 (Student's *t* test).

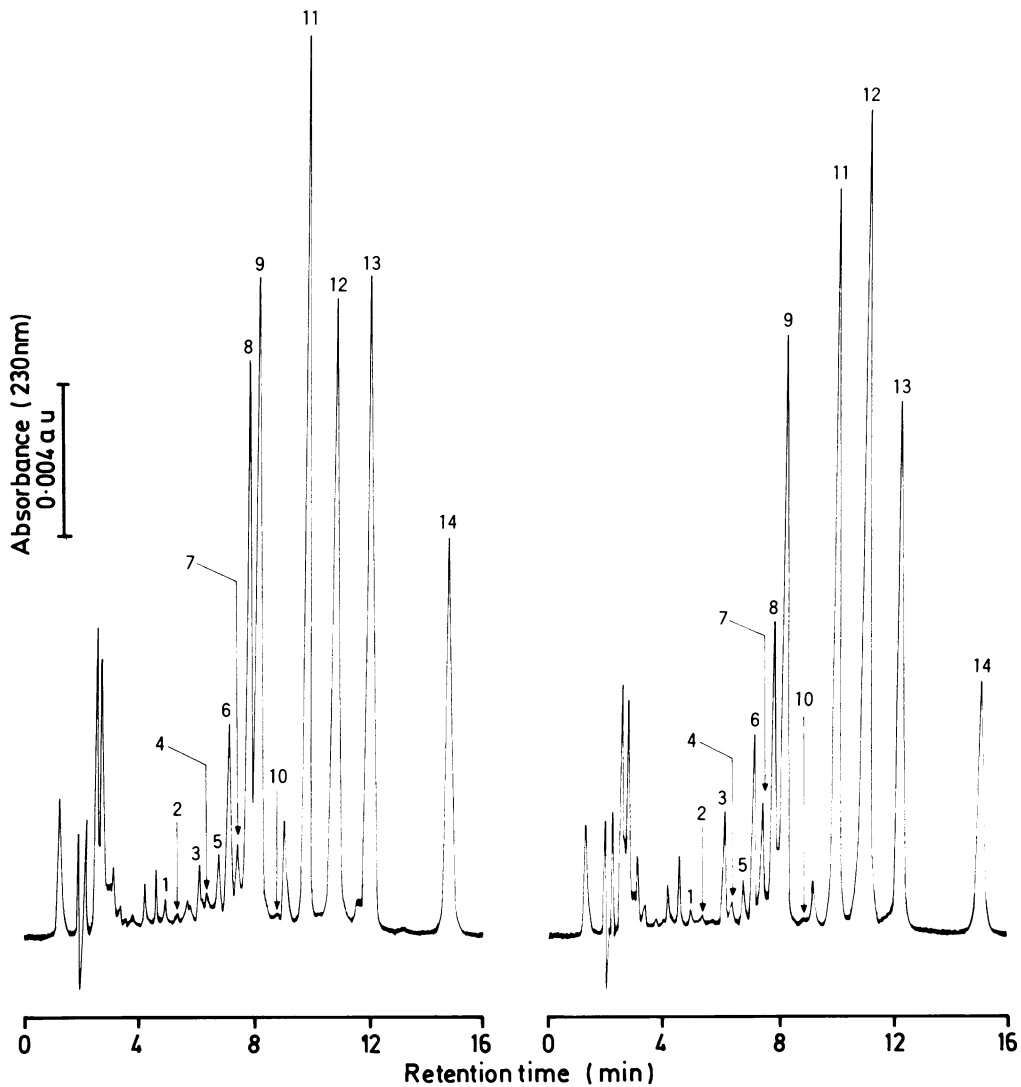


Fig. 1 HPLC profiles of 2-nitrophenylhydrazine derivatives of long chain fatty acids of phospholipids in inflamed colonic mucosa obtained from a patient with active ulcerative colitis (left) and normal colonic mucosa from a healthy control (right). Peaks correspond to individual fatty acids as follows: (1) lauric acid ($C_{12:0}$), (2) myristoleic acid ($C_{14:1}$), (3) eicosapentaenoic acid ($C_{20:5}$), (4) linolenic acid ($C_{18:3}$), (5) myristic acid ($C_{14:0}$), (6) docosahexaenoic acid ($C_{22:6}$), (7) palmitoleic acid ($C_{16:1}$), (8) arachidonic acid ($C_{20:4}$), (9) linoleic acid ($C_{18:2}$), (10) dihomo- γ -linolenic acid ($C_{20:3}$), (11) palmitic acid ($C_{16:0}$), (12) oleic acid ($C_{18:1}$), (13) margaric acid ($C_{17:0}$) (internal standard), (14) stearic acid ($C_{18:0}$).

and Smith¹³ have noted that tissue levels of active kallikrein which releases kinin and therefore activates phospholipases are increased in patients with active ulcerative colitis. Accordingly, the importance of phospholipases can hardly be denied.

One possible explanation for our observation is the increased number of inflammatory cells in the inflamed colonic mucosa, as these cells present in peripheral blood are known to contain high propor-

tions of esterified arachidonic acid.^{15,16} The cell type responsible for the increased tissue levels of esterified arachidonic acid composition was not specifically investigated in the present study, but light microscopic examination of the inflamed colonic mucosa showed a three fold increase in the cell density of inflammatory infiltrate in the lamina propria. This finding is in keeping with observations by Miyazaki *et al*¹⁸ who isolated mononuclear cells from colonic

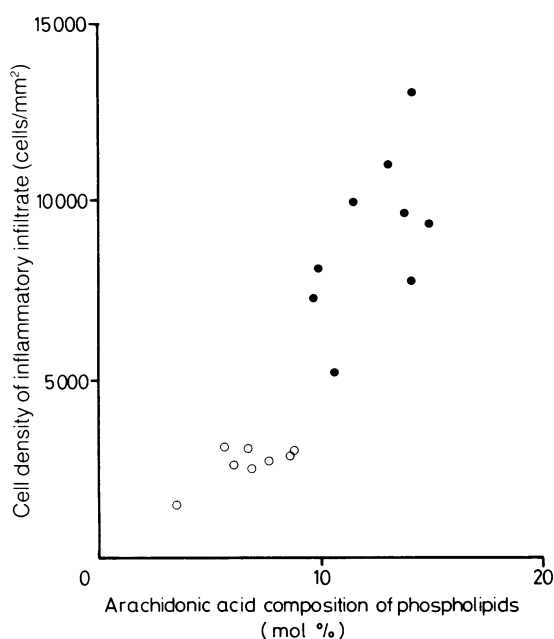


Fig. 2 Relationship between the cell density of inflammatory infiltrate in the lamina propria and the arachidonic acid composition of phospholipids in colonic mucosa in nine patients with active ulcerative colitis (closed circles) and eight healthy controls (open circles).

biopsy specimens of patients with active ulcerative colitis by the enzymatic technique. In the present study, the cell density of inflammatory infiltrate in the lamina propria correlated with the arachidonic acid composition of phospholipids in colonic mucosa. Our results suggest that in patients with active ulcerative colitis the cell density of inflammatory infiltrate may play an important role in the quantitative regulation of tissue levels of esterified arachidonic acid composition in colonic mucosa. Whether there is a difference in the arachidonic acid composition of phospholipids between the inflammatory cells observed in the inflamed colonic mucosa, however, and those in the intact mucosa remains to be assessed. Additionally, it would be of interest to know if inflammation leads to an alteration in the long chain fatty acid composition of phospholipids of the epithelial cells.

On the other hand, in the inflamed colonic mucosa from patients with active ulcerative colitis, both oleic acid and palmitoleic acid were decreased, whereas docosahexaenoic acid was increased. These results indicate another possibility that in patients with active ulcerative colitis the former two unsaturated fatty acids substitute for the excessively incorporated arachidonic acid. This may result from the difference in the rate of initial esterification *via* acyl-

transferases^{19,20} and the complex interaction of deacylation-reacylation in phospholipids.^{21,22}

In the present study, there was no significant difference in the proportions of individual saturated fatty acids of phospholipids between the inflamed and normal colonic mucosa. Moreover, the ratio of unsaturated fatty acids to saturated fatty acids did not differ significantly between the two groups. These observations suggest that in patients with active ulcerative colitis saturated fatty acids stored as phospholipids in colonic mucosa may be affected by inflammation to an extent lesser than unsaturated fatty acids. This may reflect that phospholipids containing saturated fatty acids have higher melting points and less molecular flexibility than their unsaturated counterparts.²³

All our patients were on sulphasalazine and in five of the nine patients, prednisolone was also used. These drugs were needed to enable remission to be induced. It is shown that sulphasalazine inhibits both the cyclooxygenase and lipoxygenase pathways,³ and that corticosteroids prevent formation of free arachidonic acid via phospholipases.²⁴ Pharmacological effects of these drugs on colonic arachidonic acid metabolism at the step of formation of esterified arachidonic acid are not clear, however.

Eicosapentaenoic acid and dihomo-*r*-linolenic acid are other known sources of both the cyclooxygenase and lipoxygenase products.²⁵ In the present study, the proportions of these two compounds were considerably lower than that of arachidonic acid, and no significant difference was seen between the inflamed and normal colonic mucosa. Our results suggest that these two compounds are less important as precursors of inflammatory mediators.

In summary, the present study indicates that inflamed colonic mucosa from patients with active ulcerative colitis contains a higher proportion of arachidonic acid of phospholipids than does the intact colonic mucosa from healthy controls. This observation may explain previous findings of increased tissue contents of arachidonic acid metabolites in homogenates of rectal specimens obtained from patients with ulcerative colitis.^{4,7} Furthermore, the results raise the possibility that the increase in the arachidonic acid composition of phospholipids in inflamed colonic mucosa may contribute to the enhanced production of arachidonic acid metabolites in patients with active ulcerative colitis, but further studies are needed to prove this.

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