Metabolism of 15-hydroxyeicosatetraenoic acid by Caco-2 cells

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Abstract Monolayers of Caco-2 cells, a human enterocyte cell line, were incubated with [1-^{14}C]15-hydroxyeicosatetraenoic acid (15-HETE), a lipid mediator of inflammation, and [1-^{14}C]arachidonic acid. Both fatty acids were taken up readily and metabolized by Caco-2 cells. [1-^{14}C]Arachidonic acid was directly esterified in cellular phospholipids and, to a lesser extent, in triglycerides. When [1-^{14}C]15-hydroxyeicosatetraenoic acid was incubated with Caco-2 cells, about 10% was directly esterified into cellular lipids but most (55%) was β-oxidized to ketone bodies, CO_2, and acetate, with very little accumulation of shorter carbon chain products of partial β-oxidation. The radiolabeled acetate generated from β-oxidation of [1-^{14}C]15-hydroxyeicosatetraenoic acid was incorporated into the synthesis of new fatty acids, primarily [^{14}C]palmitate, which in turn was esterified into cellular phospholipids, with lesser amounts in triglycerides. Caco-2 cells were also incubated with [5,6,8,9,11,12,14,15-^{3}H]15-hydroxyeicosatetraenoic acid; most of the radiolabel was recovered either in ketone bodies or in [^{3}H]palmitate esterified in phospholipids and triglycerides, demonstrating that most of the [^{3}H]15-hydroxyeicosatetraenoic acid underwent several cycles of β-oxidation. The binding of both 15-hydroxyeicosatetraenoic acid and arachidonic acid to hepatic fatty acid binding protein, the only fatty acid binding protein in Caco-2 cells, was measured. The K_d (6.0 μM) for 15-HETE was threefold higher than that for arachidonate (2.1 μM). —**Riehl, T. E., N. M. Bass, and W. F. Stenson.** Metabolism of 15-hydroxyeicosatetraenoic acid by Caco-2 cells. *J. Lipid Res.* 1990. **31:** 773–780.

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15(S)-Hydroxy-5,8,11,13-eicosatetraenoic acid (15-HETE) is a metabolite of arachidonic acid through the 15-lipoxygenase pathway in neutrophils (1), mast cells (2), eosinophils (3), and reticulocytes (4). 15-HETE regulates lymphocyte mitogenesis, promotes neovascularization, and stimulates migration of capillary endothelial cells (5–7). 15-HETE also inhibits a number of enzymes important in eicosanoid metabolism including cyclooxygenase, and 12-lipoxygenase (8, 9).

Studies of 15-HETE metabolism in other cell types have shown that it can be metabolized by several pathways. In J774.2 cells, a mouse macrophage-like cell line, the metabolic fate is direct esterification into cellular phospholipids and triglycerides (10). In vascular endothelial cells 15-HETE is metabolized by a mixture of esterification and partial β-oxidation (11). In neutrophils 15-HETE is metabolized to 5,15-diHETE by action of 5-lipoxygenase (12). Finally, 15-HETE is converted to the 15-keto compound by lung prostaglandin dehydrogenase in a cell-free system (13).

There is no evidence that human enterocytes synthesize 15-HETE, but enterocytes in patients with inflammatory bowel disease are exposed to high concentrations of 15-HETE; concentrations on the order of 100–300 ng of 15-HETE per gram of wet weight were found in the mucosa of surgical resections of patients with inflammatory bowel disease (14). Those studies did not define how much of the 15-HETE was intracellular and how much extracellular. The likely sources of the 15-HETE found in inflammatory bowel disease mucosa are the neutrophils, mast cells, and eosinophils which are prominent components of the inflammatory infiltrate (15).

Caco-2 cells are an enterocyte cell line derived from a human colonic adenocarcinoma but functionally and histologically resembling human ileal epithelium (16–18). We have chosen to assess 15-HETE metabolism by Caco-2 cells as a model of human intestinal epithelium, because the human intestinal epithelium is exposed to high concentrations of 15-HETE in inflammatory bowel disease. Also, it is of particular interest to study 15-HETE metabolism in a cell such as the enterocyte that is actively involved in the synthesis and excretion of complex lipids.

Abbreviations: BSA, bovine serum albumin; L-FABP, liver fatty acid-binding protein; DME, Dulbecco's Modified Eagle's medium; TLC, thin-layer chromatography; 15-HETE, 15-hydroxyeicosatetraenoic acid; HPLC, high performance liquid chromatography; RP, reversal phase.

MATERIALS AND METHODS

Chemicals

Dulbecco's Modified Eagle's medium (DME) was obtained from Cellgro and fetal bovine serum was from Cell Culture Laboratories. Caco-2 cells were a gift from Dr. Jeffrey Field, Department of Medicine, University of Iowa, Iowa City. [1-^{14}C]Arachidonic acid (58.3 Ci/mol) and [5,6,8,9,11,12,14,15-^{3}H]15-HETE (213 Ci/mmol) were obtained from Amersham. [1-^{14}C]Oleic acid (56 mCi/mmol) was obtained from New England Nuclear. [1-^{14}C]15-HETE was biosynthesized by the incubation of [1-^{14}C]arachidonic acid with soybean lipoxidase followed by reduction with triphenylphosphine (10). All other chemicals and solvents were high purity commercial materials obtained from Sigma Chemical Co and Aldrich Chemical.

Culturing of cells

Caco-2 cells were grown to 12 days post-confluence at 37°C in a 10% CO_2 atmosphere (16–18). Cells were grown as monolayers on 38-mm Millicell H. A. filters (Millipore Corp.) secured to lucite rings and rested on glass beads in 45-mm petri dishes. The area contained by the filter and ring and bathing the apical surface of the Caco-2 cells is designated as the inner well; the area outside the ring and bathing the underside of the filter is designated as the outer well. Cells were maintained in DME medium with 20% fetal calf serum. When cells were to be used in a radiolabeling experiment, the medium was replaced with serum-free medium and cells were maintained in this overnight.

Preparation of radiolabeled media

Radiolabeled medium was prepared by incubating the radiolabeled fatty acid in serum-free medium containing fatty acid-free bovine serum albumin (BSA) (1 mg/ml) at 37°C for 30 min to allow the fatty acid to bind to albumin. To initiate an experiment, the medium in the inner well (2 ml) was replaced with an equal volume of medium containing the radiolabeled fatty acid plus albumin (1 mg/ml), and the medium in the outer well (5 ml) was replaced with an equal volume of medium containing albumin (1 mg/ml) but without the radiolabeled fatty acid.

Harvesting of media and cells

After incubation of the Caco-2 cells with the radiolabeled fatty acid, the medium was collected separately from the inner and outer wells of each replicate. Cells were harvested by scraping with a flat-edged spatula and transferring them to a glass centrifuge tube. The filter was rinsed with 1 ml distilled water and these cells were combined with the first cells. The cell suspension was kept on ice and was homogenized by sonication for 15 sec with a Vibra Cell Sonicator (Sonics and Materials Inc., Danbury CT). An aliquot of homogenate was transferred to 10 ml scintillation fluid for counting of radioactivity in a Beckman LS 3801 Liquid Scintillation Counter.

Analysis of cell lipid extract

The cell homogenate was extracted by the method of Bligh and Dyer (19). An aliquot of the chloroform-extractable lipids was separated by thin-layer chromatography (TLC) using solvent system 1 (hexane–ethyl ether–acetic acid 80:20:1 (v/v/v)), followed by autoradiography. The remainder of the chloroform-extractable lipids was separated on a 0.8 cm × 17 cm silicic acid (BiopSil A100–200 mesh) column eluted sequentially with chloroform, acetone, and methanol. The chloroform fraction is designated as the neutral lipid fraction, and the methanol fraction is designated as the phospholipid fraction. An aliquot of the neutral lipid fraction, in turn, was separated on similar silicic acid column eluted sequentially with 95% hexane–5% ethyl ether, 92% hexane–8% ethyl ether, and 100% ethyl ether. The 95% hexane–5% ethyl ether fraction is designated as the triglyceride fraction, and the 100% ethyl ether fraction is designated as the free fatty acid fraction. Fractions were collected and assayed for radioactivity.

An aliquot of the triglyceride fraction was subjected to base hydrolysis (20). Ether extracts containing the released radiolabeled free fatty acids were dried under a stream of nitrogen, taken up in ethyl ether, and separated by TLC using solvent system 2 (toluene–ethyl ether–ethanol–acetic acid 50:40:2:0.2 (v/v/v/v)), followed by autoradiography. This allowed for the separation and quantification of the radiolabeled hydroxylated and nonhydroxylated free fatty acids that were released from triglyceride.

The remainder of the triglyceride fraction was used to identify and quantify the different radiolabeled fatty acid classes esterified in triglycerides. The triglyceride fraction was subjected to base methanolysis (20), and the released fatty acid methyl esters were dried under a stream of nitrogen and redissolved in chloroform. Fatty acid methyl esters were separated on the basis of the number of carbon–carbon double bonds by argentation TLC using solvent system 3 (chloroform–methanol 99:1 (v/v)) to resolve methyl esters containing 0 to 3 double bonds, and solvent system 4 (chloroform–methanol–water 80:20:2 (v/v/v)) to resolve methyl esters containing 4 to 6 double bonds (21). Fatty acids were identified by autoradiography and quantified as described above. Bands representing different classes of fatty acids were scraped from the argentation of TLC plates, eluted with chloroform–methanol 1:1 (v/v), and separated into individual fatty acids by reverse phase HPLC eluted with 80% aqueous acetonitrile (22).

The phospholipid fraction from the silicic acid column was treated as follows. An aliquot was separated on a two-dimensional TLC system (20) to separate the individual phospholipid subclasses. The plate was stained for phosphate with molybdenum blue. Radiolabeled phospholipid subclasses were identified and quantitated by autoradiography.

A second aliquot of the phospholipid fraction was subjected to base hydrolysis. TLC with solvent system 2 was used to separate the released fatty acids into hydroxylated and nonhydroxylated fractions as described above. A third aliquot was subjected to base methanolysis, argentation TLC, and reverse phase HPLC as described above for the treatment of the triglyceride extract.

In the experiments with [^{3}H]15-HETE a portion of the phospholipid fraction was subjected to base hydrolysis and the liberated fatty acids were separated into individual fatty acids by reverse phase HPLC using a 4.6 mm × 25 cm Beckman Ultrasphere column eluted with a linear gradient from 27 to 100% acetonitrile in water adjusted to pH 3 with phosphoric acid (23).

Analysis of lipids in the media

An aliquot of the postincubation medium was extracted by the method of Bligh and Dyer (19). The lipid extract was dried under a stream of nitrogen and redissolved in chloroform and an aliquot was assayed for radioactivity. The remainder of the extract was separated on TLC by solvent system 2 to separate hydroxylated from nonhydroxylated fatty acids. In the experiments with [^{3}H]15-HETE at portion of the medium extract was separated by reverse phase HPLC using a 4.6 mm × 25 cm Beckman Ultrasphere column eluted with a linear gradient from 27 to 100% acetonitrile in water adjusted to pH 3 with phosphoric acid.

Ketone body measurements

Absolute ethanol (10 ml) was added to the postincubation medium (2 ml) and left on ice for 30 min. Ethanol denatures the BSA, dissociating it from 15-HETE remaining in the medium. The mixture was centrifuged at 10,000 rpm for 15 min and the clear supernatant was decanted. The supernatant was dried in a Vortex-Evaporator and taken up in 3 ml methanol. An excess of $NaBH_4$ was added to reduce any acetoacetate to β-hydroxybutyrate. This reaction was allowed to proceed overnight at room temperature. Excess $NaHCO_3$ was added to convert β-hydroxybutyrate to its sodium salt. The methanol was evaporated under a stream of N_2, and the residue was taken up in 1.0 ml of phenacyl bromide (0.1 mmol/ml) and crown ether (0.005 mmol/ml) in acetonitrile to form the phenacyl esters of β-hydroxybutyrate and 15-HETE. Additional acetonitrile (2 ml) was added and the mixture was heated at 80°C while stirring for 30 min. The mixture was centrifuged at 1,800 rpm for 10 min and the supernatant was dried under a stream of N_2. The residue was taken up in 2 ml methanol and an aliquot was counted for radioactivity. A second aliquot was taken directly for separation on RP-HPLC, using a C_{18} analytical column. An isocratic mobile phase of 40% methanol in water was run at 1.0 ml/min for the first 20 min, followed by a 60 min linear gradient up to 100% methanol. The phenacyl ester of β-hydroxybutyrate elutes at 12 min and the phenacyl ester of 15-HETE elutes at 72 min.

$^{14}CO_2$ measurements

Open petri dishes containing Caco-2 cell monolayers on 38-mm Millicell HA filters were placed individually into closed glass CO_2 collection chambers and shaken in an incubator at 37°C for various times. After this incubation, 500 μl 10% perchloric acid was injected through a rubber septum directly into the inner well. Hyamine hydroxide (200 μl) was injected through the rubber septum onto a rolled strip of Whatman filter paper contained in an open cup suspended above the cells inside the CO_2 chamber. The chamber was incubated for an additional 90 min and the filter paper was then transferred to 10 ml scintillation fluid and counted for radioactivity. The efficiency of the $^{14}CO_2$ assay was assessed by measuring the $^{14}CO_2$ evolved from a standard solution of $NaH^{14}CO_3$. The efficiency of the assay was 58%. The total amount of CO_2 produced was calculated from the $^{14}CO_2$ and the specific activity of the radiolabeled fatty acid in the incubation medium and expressed as nmol and % of recovered radioactivity (24).

Fatty acid binding protein studies

Liver fatty acid-binding protein (L-FABP) was purified and delipidated as previously described (25, 26). Binding of 15-HETE and arachidonic acid to L-FABP was determined by competitive inhibition of [1-^{14}C]oleate binding with the use of a modification (27) of the procedure of Glatz and Veerkamp (26). Competition assays were performed in 0.46 ml 0.1 M potassium phosphate buffer, pH 7.4, containing 0.42 μM L-FABP and 0.44 μM [1-^{14}C]oleic acid. Unlabeled 15-HETE or arachidonic acid in 5 μl absolute alcohol–propylene glycol 1:3 (v/v) was added to assay tubes to obtain a range of final concentrations between 0.03 μM and 13.5 μM. After incubation at 37°C for 10 min, tubes were chilled on ice for 3 min, and 0.2 ml of a 1:1 (v/v) suspension of Lipidex 1000 (Packard Industries) in assay buffer was added to each tube with mixing. Tubes were kept on ice for 10 min and then spun at 12,200 *g* for 2 min. An aliquot of the supernatant (bound fraction) was removed for counting of radioactivity. All determinations were made in duplicate and values for bound radiolabeled oleic acid were corrected for supernatant radioactivity in the absence of protein. Protein-blank values for [1-^{14}C]oleate showed no change with either 15-

HETE or arachidonic acid over the range of concentrations studied.

Fifty percent inhibition of [1-^{14}C]oleic acid binding was derived from nonlinear, least-squares computer fitting of the competition curves; inhibition constants (K_i) were calculated (28), assuming a dissociation constant (K_d) for oleic acid binding to L-FABP of 0.9 μM (27).

RESULTS

Cellular uptake of [1-^{14}C]15-HETE and [1-^{14}C]arachidonic acid

Caco-2 cells took up [1-^{14}C]15-HETE and [1-^{14}C]arachidonic acid through their apical surface in a time-dependent manner (**Fig. 1**). After 1 h, 15-HETE uptake leveled off at 4.75 μmol per filter, whereas uptake of arachidonic acid continued to increase, and by 4 h had reached 8.2 μmol per filter.

The amount of radioactivity in the cells underestimates the total amount of radiolabeled fatty acid taken up by the cells. In the case of [^{14}C]15-HETE, much of the fatty acid underwent β-oxidation and the radioactivity was released back into the media in the form of $^{14}CO_2$ and ^{14}C-labeled ketone bodies (see Table 1). When the amount of radioactivity in these products of β-oxidation in the media is added to the radioactivity in the cells, the uptake of 15-HETE at 4 h increases to 8.8 μmol per filter, which is similar to the uptake or arachidonate at that time (8.2 μmol/filter).

Comparison of [1-^{14}C]15-HETE and [1-^{14}C]arachidonic acid metabolism

A comparison of the metabolism of [1-^{14}C]15-HETE and [1-^{14}C]arachidonic acid by Caco-2 cells after 4 h incubation is shown in **Table 1**. Cells (4×10^6 per 38-mm filter) were incubated with 6 μM of one of these ^{14}C-labeled fatty acids. With both fatty acids, more of the radiolabel was in the cells than in the media after 4 h. Similar portions of the [1-^{14}C]arachidonate (26%) and the [1-^{14}C]15-HETE (23%) remained unmetabolized in the medium.

The major metabolic fate of [1-^{14}C]arachidonate was esterification into cellular phospholipids and, to a lesser extent, triglycerides. Less than 1% of the radiolabel was recovered in CO_2 or ketone bodies; thus, very little of the added [1-^{14}C]arachidonate underwent β-oxidation. In contrast, after incubation of [1-^{14}C]15-HETE with Caco-2 cells, 15% of the radiolabel was recovered in ketone bodies and 5% in $^{14}CO_2$, indicating active β-oxidation. Unexpectedly, analysis of the ^{14}C-labeled fatty acids esterified into cellular phospholipids and triglycerides after incubation with [1-^{14}C]15-HETE revealed that although some of the fatty acids were hydroxylated, more of them were not.

We next sought to identify the radiolabeled nonhydroxylated fatty acids esterified into triglycerides and phospholipids in the cells incubated with [1-^{14}C]15-HETE. Radiolabeled fatty acid methyl esters were released from phospholipids and triglycerides by base methanolysis and separated by degree of unsaturation by argentation TLC (**Table 2**). Most of the radiolabeled nonhydroxylated fatty acids in both the triglycerides and phospholipids were saturated, with almost all of the rest monounsaturated. The saturated fatty acid methyl esters were eluted from the TLC plate and separated by reverse phase HPLC (**Fig. 2**). Essentially all of the radiolabeled saturated fatty acid was palmitate. Thus, when Caco-2 cells were incubated with [1-^{14}C]15-HETE, a large portion of the radiolabel was found in palmitate, most of which was esterified in cellular phospholipids and triglycerides. The only likely explanation for this finding is that most of the [1-^{14}C]15-HETE undergoes at least one cycle of β-oxidation with the production of [^{14}C]acetate. The [^{14}C]acetate in turn, is used in the synthesis of new fatty acids, primarily palmitate, the product of fatty acid synthetase. Most of the newly synthesized fatty acids are incorporated into phospholipids and triglycerides.

When the percentage of radiolabel incorporated in nonhydroxylated fatty acids (mainly palmitate) is added to the percentages of radiolabel in $^{14}CO_2$ and ketone bodies, the percentage of total radiolabel in products of β-oxidation after incubation of Caco-2 cells with [1-^{14}C]15-HETE is 55% (Table 1) compared with less than 1% after incubation with [1-^{14}C]arachidonate. The data presented in Table 1 indicate that the major metabolic fate of [1-^{14}C]arachidonate is esterification into phospholipids and triglycerides, whereas the major metabolic fate of [1-^{14}C]15-HETE is β-oxidation; 55% of all the molecules of

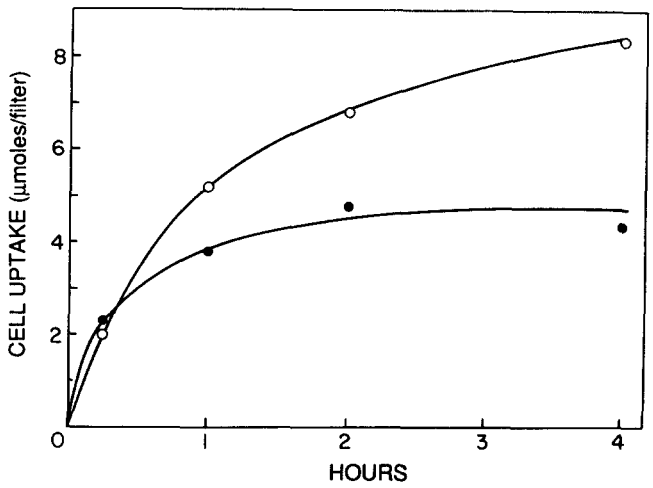

Fig. 1. Cellular uptake of 15-HETE and arachidonic acid. Caco-2 cells were incubated for up to 4 h at 37°C in DME media containing bovine serum albumin (1 mg/ml), and either: (○)[1-^{14}C]arachidonic acid (6.0 μM) or (●) [1-^{14}C]15-hydroxyeicosatetraenoic acid (6.0 mM). Data are presented as μmol/filter.

TABLE 1. Metabolism of [1-^{14}C]15-HETE and [1-^{14}C]arachidonic acid by Caco-2 cells after 4 h incubation

	[1-^{14}C]15-HETE		[1-^{14}C]Arachidonic Acid	
Media				
$^{14}CO_2$	5[a]	(5)[b]	0.12[a]	(0.1)[b]
Ketone bodies	14	(15)	0.17	(0.2)
Lipids				
Hydroxylated fatty acids	21	(23)		
Nonhydroxylated fatty acids			22	(26)
Polar lipids	4	(4)	1	(1)
Cells				
Free fatty acids				
Hydroxylated fatty acids	7	(8)		
Nonhydroxylated fatty acids	2	(2)	4	(5)
Triglycerides				
Hydroxylated fatty acids	1	(1)		
Nonhydroxylated fatty acids	9	(10)	2	(2)
Phospholipids				
Hydroxylated fatty acids	9	(10)		
Nonhydroxylated fatty acids	21	(23)	57	(66)
Total recovered radioactivity	93	(100)	86	(100)
Products of β-oxidation	51	(55)	0.36	(0.3)

Caco-2 cells were incubated for 4 h at 37°C in Dulbecco's Modified Eagle's medium (DME) containing bovine serum albumin (1 mg/ml) and [1-^{14}C]15-HETE (6 μM) or [1-^{14}C]arachidonic acid (6 μM).

[a] nmoles.

[b] % recovered radioactivity (in parentheses).

[1-^{14}C]15-HETE undergoes at least one cycle of β-oxidation. In addition, a smaller portion of the [1-^{14}C]15-HETE is directly incorporated into phospholipids.

Metabolism of [^{3}H]15-HETE

Studies of the metabolism of [1-^{14}C]15-HETE reflect the metabolic fate of the carboxy terminal carbon atom. These studies demonstrate that most molecules of 15-HETE undergo at least one cycle of β-oxidation but give no information about the metabolic fate of the remainder of the molecule. To address this issue, Caco-2 cells were incubated with [5,6,8,9,11,12,14,15-^{3}H]15-HETE. After a 4-h incubation of Caco-2 cells with [^{3}H]15-HETE, the major radiolabeled metabolites were ketone bodies in the medium (10% of total counts) and nonhydroxylated fatty acids esterified into cellular PL (15%) (**Table 3**). The lipid extract of the medium was separated by reverse phase HPLC. This separation revealed that the major lipid-extractable radiolabeled compound in the medium was [^{3}H]15-HETE with much smaller amounts of two other compounds that eluted at 22 and 26 min (**Fig. 3**, panel A). Separation of the lipid extract of the media by TLC (solvent system I) revealed that these two compounds comigrate with 15-HETE, suggesting that they are hydroxy fatty acids. These two compounds together constitute 20% of lipid-extractable counts in the media and 3% of total recovered counts. They may represent shorter carbon chain length hydroxylated fatty acids formed as the result of [^{3}H]15-HETE undergoing a limited amount (two to four cycles) of β-oxidation.

TABLE 2. Classes of radiolabeled fatty acids esterified into phospholipids and triglycerides in Caco-2 after 4 h incubation with [1-^{14}C]15-HETE

	Phospholipids		Triglycerides	
	nmol	*% recovered radioactivity*	*nmol*	*% recovered radioactivity*
Nonhydroxylated fatty acid				
Saturated	13.5	15.2	4.8	5.1
Monounsaturated	6.1	6.8	3.2	3.5
Diunsaturated	1.4	1.5	1.0	1.1
Hydroxylated fatty acid				
15-HETE	9.0	9.9	1.0	1.1

Caco-2 cells were incubated for 4 h at 37°C in DME media containing bovine serum albumin (1 mg/ml) and [1-^{14}C]15-HETE (6 μM). Mixtures of fatty acid methyl esters were liberated from phospholipids and triglycerides by base methanolysis, and separated according to the degree of unsaturation by argentation TLC.

The cells were extracted and the lipid extract was separated by silicic acid chromatography. Most of the lipid-extractable radioactivity in the cells was in the phospholipid fraction. The phospholipid fraction was subjected to base hydrolysis and the released fatty acids were separated by reverse phase HPLC (Fig. 3B). The major radiolabeled fatty acid was palmitate with lesser amounts of 15-HETE. There were also two unidentified fatty acids eluting after 15-HETE but before palmitate. The major metabolic fate of [^{3}H]15-HETE is β-oxidation.

Fatty acid binding protein

Clearly Caco-2 cells metabolize 15-HETE and arachidonate by different pathways but the mechanism that

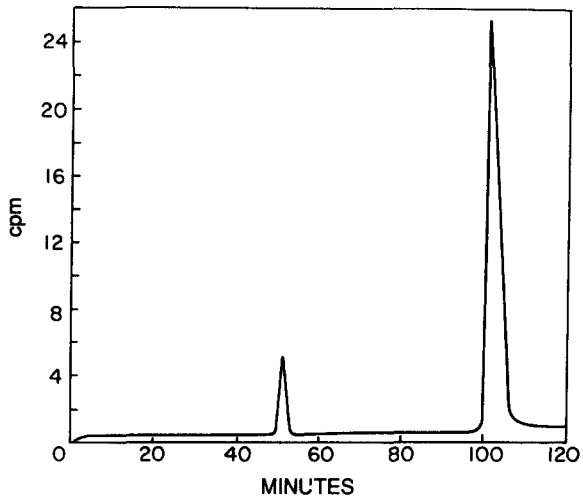

Fig. 2. Elution profile of reverse phase HPLC separation of saturated fatty acid methyl esters. Caco-2 cells were incubated with [1-^{14}C]15-HETE. The lipids were extracted and separated by silicic acid chromatography. The phospholipid fraction from the silicic acid column was subjected to base methanolysis, and the released fatty acid methyl esters were separated by argentation TLC. The argentation TLC band containing saturated fatty acid methyl esters was eluted and separated by HPLC.

directs these two fatty acids in different directions remains unclear. FABPs are present in many cell types and some investigators have suggested that these proteins not only bind fatty acids but also direct fatty acids to specific intracellular destinations (29, 30). The only FABP present in Caco-2 cells in L-FABP (personal communication, Dr. Jeffrey Gordon, Washington University). We measured the binding of 15-HETE and arachidonic acid to L-FABP to determine whether this protein may play a role in directing their metabolic fate. 15-HETE binding to L-FABP was determined by competitive displacement of oleate (**Fig. 4**). The K_i for 15-HETE, corrected for the concentration of oleate in the assay and for the K_d for oleate (0.9 μM), was 6.0 μM. This is also a reasonable approximation of the K_d for 15-HETE, and K_i for arachidonic acid of 2.1 μM obtained using the same competitive inhibition approach.

DISCUSSION

This study demonstrates that Caco-2 cells metabolize the majority of added 15-HETE by β-oxidation with a smaller but significant portion of 15-HETE being esterified directly into cellular phospholipids and triglycerides. Earlier studies of 15-HETE metabolism in mouse peritoneal macrophages demonstrated that some 15-HETE was taken up by the cells and esterified into phospholipids and triglycerides, but much more was metabolized to somewhat more polar compounds than were found in the media. These compounds were not identified but the authors speculated that they might be dihydroxy fatty acids (30). Studies of 15-HETE metabolism by a mouse macrophage-like cell line demonstrated that incorporation into phospholipids, specifically phosphatidylinositol, was its major metabolic fate (10). Incubation of 15-HETE with cultured bovine aorta endothelial cells and human umbilical vein endothelial cells resulted in incorporation of some 15-HETE into phospholipids, but most underwent two cycles of β-oxidation with production of 11-hydroxyhexadecatrienoic acid [16:3(11-OH)] which was found in the medium (11). In a similar study vascular smooth muscle cells metabolized 12(S)-hydroxy-5,8,10,14-eicosatetraenoic acid by two cycles of β-oxidation to yield 8-hydroxyhexadecatrienoic acid (23). In neither case was it clear why β-oxidation stopped after two cycles.

There are two striking differences between the present study and earlier studies of 15-HETE metabolism. The first is that in Caco-2 cells 15-HETE undergoes more cycles of β-oxidation than reported in other cell types. Our studies with [5,6,8,9,11,12,14,15-^{3}H]15-HETE indicate that most of the 15-HETE underwent at least eight cycles of β-oxidation (the number of cycles necessary to reach the fifteenth carbon atom, the last carbon substituted with tritium).

In contrast to the studies of 15-HETE metabolism by endothelial cells in which β-oxidation stopped after two cycles, there was little evidence for such limited β-oxidation in Caco-2 cells. The two small peaks in Fig. 3A may represent products of limited β-oxidation, but these

TABLE 3. Metabolism of [^{3}H]15-HETE by Caco-2 cells after 4 h incubation

	[^{3}H]15-HETE	
Media		
Ketone bodies	1.2[a]	(10)[b]
Hydroxylated fatty acids	7.7	(59)
Polar lipids	0.3	(2)
Cells		
Free fatty acids		
Hydroxylated fatty acids	0.4	(3)
Nonhydroxylated fatty acids	0.2	(2)
Triglycerides		
Hydroxylated fatty acids	0.4	(3)
Nonhydroxylated fatty acids	0.3	(0.2)
Phospholipids		
Hydroxylated fatty acids	1	(8)
Nonhydroxylated fatty acids	2	(15)
Total recovered radioactivity	13.1	(100)
Products of β-oxidation	3.4	(26)

Caco-2 cells were incubated for 4 h at 37°C in DME media containing bovine serum albumin (1 mg/ml) and [5,6,8,9,11,12,14,15,-^{3}H)15-hydroxyeicosatetraenoic acid (1 μM).

[a] nmoles.

[b] % recovered radioactivity (in parentheses).

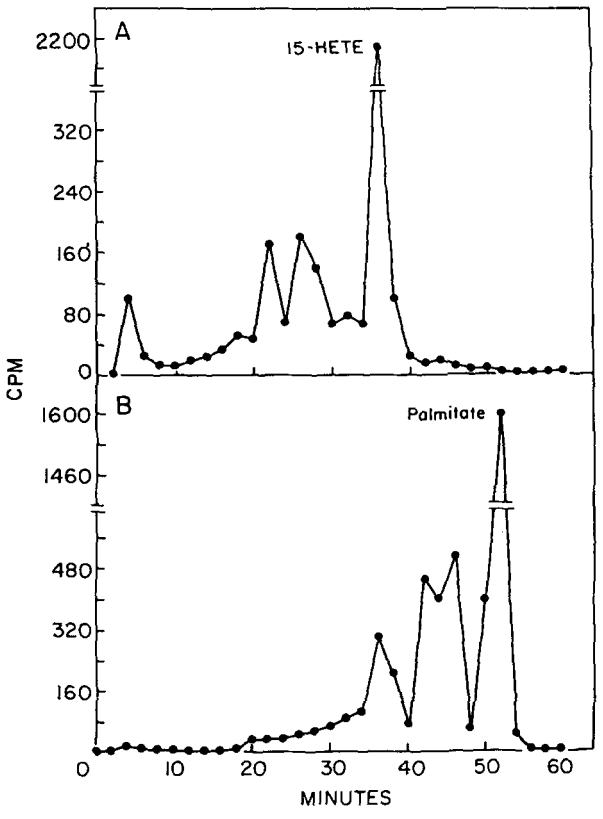

Fig. 3. Reverse phase HPLC elution profiles of fatty acids contained in the media (A) and in cellular phospholipids (B) of Caco-2 cells incubated for 4 h at 37°C in DME media containing bovine serum albumin (1 mg/ml) and [5,6,8,9,11,12,13,14-^{3}H]15-hydroxyeicosatetraenoic acid (1 μM). Panel A: the media was removed and extracted; the lipid extract was separated by reverse phase HPLC eluted (with a linear gradient from 27 to 100% acetonitrile in water adjusted to pH 3 with phosphoric acid) over 50 min. Panel B: the cells from the above incubation were extracted and the phospholipid fraction was isolated from the lipid extract by silicic acid chromatography. The phospholipid fraction was subjected to base hydrolysis and the mixture of liberated fatty acids was separated by the HPLC system described for the media extract.

peaks contain only 3% of the total recovered radioactivity. It is not clear why β-oxidation of 15-HETE should be more extensive in Caco-2 cells than in vascular endothelial and smooth muscle cells. The more extensive β-oxidation of 15-HETE by Caco-2 cells is more consistent with the usual experience of complete β-oxidation of long chain polyunsaturated fatty acids in most cell types. Even if the *cis* double bond or the hydroxy group blocked further β-oxidation, one would expect 15-HETE to undergo six cycles of β-oxidation.

The second striking difference between Caco-2 cells and other cell types in their metabolism of 15-HETE is that in Caco-2 cells the major product of 15-HETE β-oxidation is acetate, which goes into the synthesis of palmitate through fatty acid synthetase. In lipogenic tissues (liver, intestinal epithelial cell, adipose tissue, mammary gland) a cytosolic acetyl CoA synthetase has been identified in addition to the mitochondrial acetyl CoA synthetase identified in other tissues (31). It is possible that the cytosolic enzyme synthesizes acetyl CoA for lipogenesis, whereas the mitochondrial acetyl CoA synthetase activates acetate headed for oxidation.

Clearly, Caco-2 cells metabolize 15-HETE and arachidonate differently. The major metabolic fate of 15-HETE is β-oxidation; direct esterification into phospholipids and triglycerides is a less important pathway. In contrast, direct esterification is the major metabolic pathway for arachidonate. The regulation of fatty acid metabolism is poorly understood and the mechanisms that direct one fatty acid towards esterification into phospholipids and triglycerides and direct another fatty acid towards β-oxidation are not defined. It has been suggested that fatty acid binding proteins may play a role in directing fatty acids towards different metabolic pathways (29, 30). Caco-2 cells contain only the hepatic fatty acid binding protein. We considered the possibility that the different metabolic fates of 15-HETE and arachidonate in Caco-2 may reflect a difference in binding to L-FABP. Although 15-HETE bound somewhat less avidly to L-FABP than arachidonic acid, it is unknown to what extent the threefold difference in binding affinity contributes to the major difference in metabolic fates.

The extensive oxidation of 15-HETE by Caco-2 is of particular interest in view of the high levels of 15-HETE found in the inflamed intestinal mucosa of patients with inflammatory bowel disease. The epithelial cells has been viewed as the primary target cell of the inflammatory process in inflammatory bowel disease. Previously, the participation of the intestinal epithelial cell in the

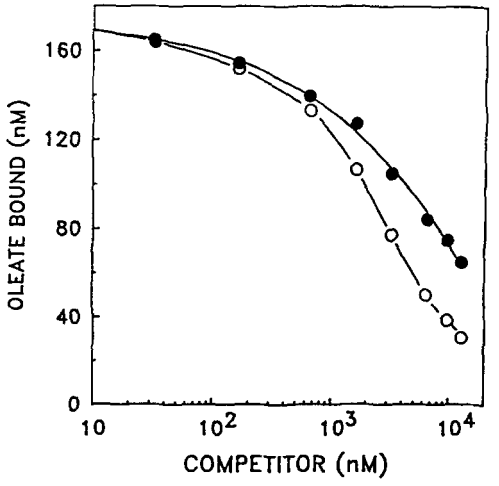

Fig. 4. Competitive inhibition of [1-^{14}C]oleic acid binding to liver FABP. Unlabeled 15-HETE [●] or arachidonic acid (○) were incubated at concentrations ranging from 0.03 to 13.5 μM with [1-^{14}C]oleic acid (0.44 μM) and L-FABP (0.42 μM). Unbound [1-^{14}C]oleic acid was separated from protein-bound fatty acid with Lipidex 1000. The displacement data were analyzed by a nonlinear, least-squares competitive inhibition curve-fitting program.

inflammatory response has been thought to be purely passive. However, the demonstration here that Caco-2 cells are able to metabolize an important lipid mediator of inflammation suggests that epithelial cells may abrogate and modify the intestinal inflammation by metabolizing mediators of inflammation.

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