

5-Lipoxygenase-mediated Endogenous DNA Damage*

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Wenyang Jian, Seon Hwa Lee, Michelle V. Williams, and Ian A. Blair¹

From the Centers for Cancer Pharmacology and Excellence in Environmental Toxicology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6160

Lipoxygenases (LOs) convert polyunsaturated fatty acids into lipid hydroperoxides. Homolytic decomposition of lipid hydroperoxides gives rise to endogenous genotoxins such as 4-oxo-2(*E*)-nonenal, which cause the formation of mutagenic DNA adducts. Chiral lipidomics analysis was employed to show that a 5-LO-derived lipid hydroperoxide was responsible for endogenous DNA-adduct formation. The study employed human lymphoblastoid CESS cells, which expressed both 5-LO and the required 5-LO-activating protein (FLAP). The major lipid peroxidation product was 5(*S*)-hydroperoxy-6,8,11,14-(*E,Z,Z,Z*)-eicosatetraenoic acid, which was analyzed as its reduction product, 5(*S*)-hydroxy-6,8,11,14-(*E,Z,Z,Z*)-eicosatetraenoic acid (5(*S*)-HETE). Concentrations of 5(*S*)-HETE increased from 0.07 ± 0.01 to 45.50 ± 4.05 pmol/10⁷ cells upon stimulation of the CESS cells with calcium ionophore A23187. There was a concomitant increase in the 4-oxo-2(*E*)-nonenal-derived DNA-adduct, heptanone-etheno-2'-deoxyguanosine (HedGuo) from 2.41 ± 0.35 to 6.31 ± 0.73 adducts/10⁷ normal bases. Biosynthesis of prostaglandins, 11(*R*)-hydroxy-5,8,12,14-(*Z,Z,E,Z*)-eicosatetraenoic acid, and 15(*R,S*)-hydroxy-5,8,11,13-(*Z,Z,Z,E*)-eicosatetraenoic acid revealed that there was cyclooxygenase (COX) activity in the CESS cells. Western blot analysis revealed that COX-1 was expressed by the cells, but there was no COX-2 or 15-LO-1. FLAP inhibitor reduced HedGuo-adducts and 5(*S*)-HETE to basal levels. In contrast, aspirin, which had no effect on 5(*S*)-HETE, blocked the formation of prostaglandins, 15-HETE, and 11-HETE but did not inhibit HedGuo-adduct formation. These data showed that 5-LO was the enzyme responsible for the generation of the HedGuo DNA-adduct in CESS cells.

matically by reactive oxygen species (ROS) (3). Arachidonic acid, one of the essential PUFAs present in cell membranes, is released from phospholipids by different phospholipase A₂ isoforms upon diverse physical, chemical, inflammatory, and mitogenic stimuli (4). The free arachidonic acid then serves as a substrate for LOs, COXs, or ROS to produce a variety of lipid hydroperoxides (5, 6). ROS, 12-LO, and 15-LO can also act directly upon arachidonic acid esterified in phospholipids to produce lipid hydroperoxides (7), which are reduced (8), hydrolyzed by phospholipase A₂ (4), and then secreted as the corresponding free HETEs (9). COX-2-mediated (10) and 15-LO-1-mediated (11) metabolism of linoleic acid results in the formation of 13(*S*)-hydroperoxy-9,11-(*Z,E*)-octadecadienoic acid, which is rapidly reduced to 13(*S*)-hydroxy-9,11-(*Z,E*)-octadecadienoic acid (HODE) and secreted from cells. Arachidonic acid is specifically metabolized by 5-LO into 5(*S*)-HpETE, which is either reduced to 5(*S*)-HETE or serves as precursor to the formation of leukotrienes (LTs) (Scheme 1) (12). In contrast, ROS-mediated reactions produce racemic mixtures of all possible regioisomers of HpETEs and 13(*S*)-hydroperoxy-9,11-(*Z,E*)-octadecadienoic acids (3) that are subsequently secreted from cells as complex mixtures of racemic HETEs and HODEs. Therefore, the ability to analyze different HETE and HODE enantiomers and regioisomers is important for elucidating specific cellular lipid peroxidation pathways (13).

The conversion of arachidonic acid to 5(*S*)-HpETE by 5-LO is critically dependent upon the presence of FLAP (14). 5-LO and FLAP are expressed primarily in inflammatory cells such as polymorphonuclear leukocytes, monocytes, macrophages, and mast cells (12, 15–17). Therefore, 5-LO-mediated LT formation is thought to play a critical role in inflammation and allergic disorders (18–21). In addition, a number of studies have implicated 5-LO-derived arachidonic acid metabolites as mediators of atherogenesis and heart disease (12, 22, 23). The 5-LO pathway of arachidonic acid metabolism has also been proposed to play a role in prostate and pancreatic cancer (24–26).

Lipid hydroperoxides undergo homolytic decomposition into bifunctional electrophiles such as 4-hydroxy-2(*E*)-nonenal, ONE, 4,5-epoxy-2(*E*)-decenal, and 4-hydroperoxy-2(*E*)-nonenal (27). These bifunctional electrophiles are highly reactive and can readily modify intracellular molecules including glutathione (GSH) (28, 29), DNA, (5, 6), and proteins (30, 31). Our previous *in vitro* studies characterized the bifunctional electrophiles ONE and 4-hydroperoxy-2(*E*)-nonenal as major

PUFAs² can be converted into lipid hydroperoxides enzymatically by the action of LOs (1) and COXs (2) or nonenzy-

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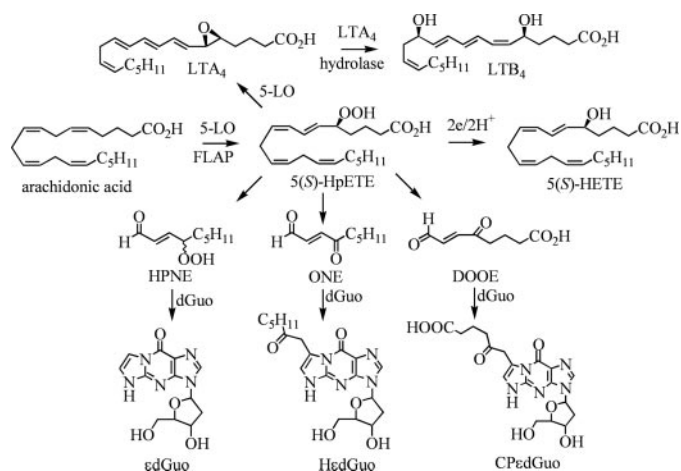
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¹ To whom correspondence should be addressed. Center for Cancer Pharmacology, University of Pennsylvania School of Medicine, 854 BRB II/III, 421 Curie Blvd., Philadelphia, PA 19104-6160.

² The abbreviations used are: PUFA, polyunsaturated fatty acid; APCI, atmospheric pressure chemical ionization; ECAPCI, electron capture APCI; COX, cyclooxygenase; dGuo, 2'-deoxyguanosine; edGuo, etheno-dGuo; HedGuo, heptanone-etheno-dGuo; CPedGuo, carboxypentane-2'-deoxyguanosine; FLAP, 5-lipoxygenase-activating protein; 5-HETE, 5-hydroxy-6,8,11,14-(*E,Z,Z,Z*)-eicosatetraenoic acid; 8-HETE, 8-hydroxy-5,9,11,14-(*Z,E,Z,Z*)-eicosatetraenoic acid; 11-HETE, 11-hydroxy-5,8,12,14-(*Z,Z,E,Z*)-eicosatetraenoic acid; 12-HETE, 12-hydroxy-5,8,10,14-(*Z,Z,E,Z*)-eicosatetraenoic acid; 15-HETE, 15-hydroxy-5,8,11,13-(*Z,Z,Z,E*)-eicosatetraenoic acid; 13-HODE, 13-hydroxy-9,11-(*Z,E*)-octadecadienoic acid; HpETE, hydroperoxyeicosatetraenoic acid; LO, lipoxygenase; LTB₄, leukotriene B₄; MOPS, 3-morpholinopropanesulfonic acid; MRM, multiple

reaction monitoring; MS, mass spectrometry; ONE, 4-oxo-2(*E*)-nonenal; PFB-Br, pentafluorobenzyl bromide; PG, prostaglandin; ROS, reactive oxygen species; HPLC, high performance liquid chromatography (LC); Bis-Tris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol.

5-Lipoxygenase-mediated Endogenous DNA Damage



SCHEME 1. 5-LO-mediated formation of arachidonic acid metabolites and dGuo-adducts. HPNE, 4-hydroperoxy-2(E)-nonenal; DOOE, dioxo-6-oc-tenoic acid.

products arising from the homolytic decomposition of 5-LO-derived 5(S)-HpETE (32). Reactions with DNA resulted in the formation of etheno-2'-deoxyguanosine (edGuo) from 4-hydroperoxy-2(E)-nonenal and heptanone-εdGuo (HedGuo) from ONE (Scheme 1). 5,8-Dioxo-6-oc-tenoic acid, a bifunctional electrophile from the carboxyl terminus of 5(S)-HpETE, gave rise to the novel DNA-adduct carboxypentanone-εdGuo (CPedGuo)-adduct as shown in Scheme 1.

Previous studies have demonstrated that lipid hydroperoxides generated by COX-2 could lead to the formation of endogenous DNA adducts in epithelial cells (6). Cellular 5-LO, like COX-2, synthesizes lipid hydroperoxides on the nuclear membrane. Therefore, it is highly possible that 5-LO could also mediate the formation of lipid hydroperoxide-derived endogenous DNA adducts in cells. CESS is a human lymphoblastic cell line that expresses both 5-LO and FLAP, and they have been used as a model for inflammatory cells to examine the role of 5-LO metabolites in signal transduction (33, 34). In the present study, CESS cells provided an ideal model to elucidate the relationship of 5-LO mediated-lipid peroxidation and DNA-adduct formation in a cellular setting. Stable isotope dilution chiral LC-electron capture (EC) APCI/MRM/MS (13) was used to monitor the concomitant formation of lipid hydroperoxides in the presence of different enzyme stimulator or inhibitors. DNA-adduct formation in the same cells was measured by a stable isotope dilution APCI/MRM/MS method. The powerful tool of chiral lipid analysis enabled us to dissect the complicated lipid peroxidation pathways and to correlate them with endogenous DNA-adduct formation. The results demonstrated that 5-LO-mediated lipid peroxidation could cause HedGuo formation in cells. This novel finding provided additional explanation for the previous observation that increased 5-LO activity was associated with cancers and cardiovascular diseases (24–26).

EXPERIMENTAL PROCEDURES

Material and Reagents—CESS cells was obtained from ATCC (Manassas, VA). RPMI 1640, fetal bovine serum, and penicillin-streptomycin were supplied by Invitrogen. BCA protein assay reagent was obtained from Pierce. Pre-cast 7% NuPAGE Novex Tris acetate gels and 0.45-μm nitrocellulose

membranes were obtained from Invitrogen. 5-LO polyclonal antiserum, 15-LO (rabbit reticulocyte) polyclonal antibody, and COX-2 (murine) polyclonal antibody were obtained from Cayman Chemical Co. (Ann Arbor, MI). COX-1 polyclonal antibody (sc-1752) was from Santa Cruz Biotechnology Inc (Santa Cruz, CA). Horseradish peroxidase-conjugated mouse anti-goat/sheep antibody and horseradish peroxidase-conjugated goat anti-rabbit antibody was from Sigma-Aldrich. The enhanced chemiluminescence (ECL) Western blotting reagent was supplied by Amersham Biosciences. 13(R)-HODE, 13(S)-HODE, 5(R)-HETE, 5(S)-HETE, 8(R)-HETE, 8(S)-HETE, 11(R)-HETE, 11(S)-HETE, 12(R)-HETE, 12(S)-HETE, 15(R)-HETE, 15(S)-HETE, LTB₄, PGE₂, PGD₂, PGF_{2α}, 13(S)-[²H₄]HODE, 5(S)-[²H₈]HETE, 12(S)-[²H₈]HETE, 15(S)-[²H₈]HETE, [²H₄]LTB₄, [²H₄]PGE₂, [²H₄]PGD₂, [²H₄]PGF_{2α} and MK886 were purchased from Cayman Chemical Co. (Ann Arbor, MI). Ammonium acetate, aspirin, calcium ionophore A23187, diisopropylethylamine, DMSO, EDTA, glycine, magnesium chloride, MOPS, Nonidet P-40, phenylmethanesulfonyl fluoride, protease mixture, sodium chloride, Tris hydrochloride, and zinc chloride were obtained from Sigma-Aldrich. DNA extraction kit was purchased from Wako Chemicals USA, Inc. (Richmond, VA). DNase I, phosphodiesterase I, and shrimp alkaline phosphatase were purchased from Calbiochem, Worthington Biochemical Corp. (Lakewood, NJ), and Roche Applied Science, respectively. Costar 0.2-μm microcentrifuge filter was provided by Corning Inc. (Corning, NY). LC-18 and LC-Si solid phase extraction tubes (1 g, 6 ml) were from Supelco (Bellefonte, PA). Acetonitrile, hexane, HPLC grade water, isopropyl alcohol, methanol, and methylene chloride were obtained from Fisher. Gases were supplied by BOC Gases (Lebanon, NJ).

Cell Culture and Stimulation—CESS cells were incubated in RPMI 1640 (10 ml) containing 10% fetal bovine serum, 100 units/ml of penicillin, and 100 μg/ml of streptomycin at 37 °C in an atmosphere of 5% CO₂. After the cells reached the density of 1 × 10⁶/ml, the complete RPMI 1640 was removed and replaced with serum-free RPMI 1640 for treatment. 1 × 10⁷ cells were treated with vitamin C (dissolved in water, final concentration of 1.0 mM) and/or A23187 (dissolved in DMSO, final concentration of 1.0 μM). Cells were incubated for 1 h, and the medium was collected for lipid analysis. The cells were then re-suspended in complete RPMI 1640 and added with vitamin C and/or A23187 again. The cells were incubated till 24 h and collected for DNA extraction. For the treatment of MK886 (dissolved in ethanol, final concentration of 1.0 μM) and aspirin (dissolved in ethanol, final concentration of 200.0 μM), the procedure was the same as above except that the cells were additionally pretreated with MK886 or aspirin for 2 h.

LO and COX Expression—Expressions of 5-LO, COX-1, COX-2, 15-LO in the cells were determined by Western blot analysis. Briefly, the cells were washed twice with ice-cold phosphate-buffered saline and suspended in lysis buffer containing 50 mM Tris (pH 8.0), 5 mM EDTA, 150 mM NaCl, 0.5% Nonidet P-40, 0.5 mM phenylmethanesulfonyl fluoride, and 1 × protease mixture for mammalian tissue. The cells were lysed on ice for 20-min and centrifuged at 10,000 × g for 10 min. The supernatant was collected, and the protein concentration was deter-

mined by BCA protein assay reagent. Cellular protein (50 μg) was loaded on pre-cast 7% NuPAGE Bis-Tris gels and then transferred to 0.45 μM nitrocellulose membranes. The blots were blocked with 5% nonfat milk in Tris-buffered saline (100 mM Tris (pH 7.5), 150 mM NaCl) containing 0.1% Tween 20 and then incubated with primary antibodies followed by reaction with secondary antibody. Protein bands were visualized with the ECL reagent.

Lipid Extraction—A portion of cell culture medium (3 ml) was collected for lipid extraction. A mixture of internal standard containing 1.0 ng of each of following compounds was added to each sample: 13(S)-[$^2\text{H}_4$]HODE, 5(S)-[$^2\text{H}_8$]HETE, 12(S)-[$^2\text{H}_8$]HETE, 15(S)-[$^2\text{H}_8$]HETE, [$^2\text{H}_4$]LTB₄, [$^2\text{H}_4$]PGE₂, [$^2\text{H}_4$]PGD₂, [$^2\text{H}_4$]PGF_{2 α} . The pH of the samples was adjusted to 3.0 with hydrochloric acid. Lipids were extracted with diethyl ether (4 ml \times 2), and the organic layer was evaporated to dryness under nitrogen. Lipids were dissolved in 300 μl of acetonitrile containing PFB-Br (10:300, v/v) and diisopropylethylamine (5:300, v/v) and heated at 60 $^\circ\text{C}$ for 1 h. The solution was evaporated to dryness under nitrogen and re-dissolved in 100 μl of hexane/ethanol (97:3, v/v). A portion of the solution (20 μl) was subjected to normal phase chiral LC/ECAPCI/MS analysis. Calibration curves were obtained by spiking 3 ml of RPMI 1640 with authentic lipid standards. The internal standards were then added, and the standard solutions underwent the same extraction and analytical procedure as for the samples. Calibration curves were calculated with a linear regression analysis of peak area ratios of authentic standards against internal standards. Production of lipid metabolites from the cells was calculated by interpolation from the calibration curve and expressed as pmol/ 10^7 cells.

Extraction and Isolation of DNA Adducts—DNA from 1×10^7 cells was extracted with Wako DNA extraction kit which employed sodium iodide as a chaotropic agent, and the procedure was described in the manufacturer's instructions. Typically, 600–800 μg of DNA was obtained. The DNA was dissolved in 1 ml of 10 mM Tris/100 mM MgCl₂ (pH 7.4). DNase I (556 units in 10 μl of 10 mM MOPS/100 mM MgCl₂) was added, and the samples were incubated at 37 $^\circ\text{C}$ for 1.5 h. The pH of the sample was then adjusted to 9.0 with 150 μl of 0.2 M glycine buffer (adjust pH to 10.0 with NaOH). Phosphodiesterase I (100 units in 10 μl of water) was added and incubated at 37 $^\circ\text{C}$ for 2 h. At the end of incubation, 150 μl of 50 mM Tris-HCl (pH 7.4) was added along with 30 units (30 μl) of shrimp alkaline phosphatase and 150 μl of $10 \times$ shrimp alkaline phosphatase reaction buffer provided by the manufacturer. The incubation was continued at 37 $^\circ\text{C}$ for 2 h, and the sample was then filtered through Costar 0.2- μm microcentrifuge filters. An aliquot (50 μl) was taken for LC/UV analysis of the base content. Quantitation of DNA bases was carried out by constructing standard curves of known amount of bases. The remaining sample solution was added with 20.0 ng of $^{15}\text{N}_5$ -HedGuo as internal standards and applied to LC-18 solid phase extraction tube (1 g, 6 ml), which was preconditioned with acetonitrile (18 ml) followed with water (18 ml). For the calibration curve, a certain amount of authentic HedGuo (0, 0.05, 0.10, 0.20, 0.50, 1.00, 2.00, 5.00 ng of each) was mixed with internal standards and subjected to the same procedure as that of samples from this step. The cartridge

was then washed with 4 ml of water and 1 ml of methanol/water (5:95, v/v). The DNA adducts were eluted with 6 ml of acetonitrile/water (1:1, v/v). The eluate was evaporated to dryness under nitrogen and then dissolved in 300 μl of acetonitrile containing PFB-Br (20:300, v/v) and diisopropylethylamine (4:300, v/v) and incubated at room temperature for 1.5 h. The solution was evaporated to dryness under nitrogen and dissolved in 100 μl of methanol/methylene chloride (25:75, v/v). The solution was applied to LC-Si solid phase extraction tube (1 g, 6 ml), which was preconditioned with methylene chloride (12 ml). The cartridge was then washed with 3 ml of methanol/methylene chloride (1:99, v/v) and 1 ml of methanol/methylene chloride (5:95, v/v). The derivatized DNA adducts were eluted with 6 ml of methanol/methylene chloride (25:75, v/v). The eluate was evaporated to dryness under nitrogen and then dissolved in 100 μl of acetonitrile, water (80:20, v/v). A portion of the solution (20 μl) was subjected to reverse phase LC/APCI/MS analysis. Calibration curves were calculated with a linear regression analysis of peak area ratios of authentic standards against internal standards. DNA adduct levels were calculated by interpolation from the calibration curve and normalized with the total base content in each sample.

LC Conditions for Lipids Analysis—Chromatography was performed using a Waters Alliance 2690 HPLC system (Waters Corp., Milford, MA). A Chiralpak AD-H column (250 \times 4.6-mm; inner diameter, 5 μm ; Daicel Industries, Ltd., Tokyo, Japan) was employed for system 1. Solvent A was hexane, and solvent B was methanol/isopropanol (1:1, v/v). The flow rate was 1.0 ml/min, and the gradient was as follows: 2% B at 0 min, 2% B at 3 min, 3.6% B at 11 min, 8% B at 15 min, 8% B at 27 min, 50% B at 30 min, 50% B at 35 min, 2% B at 37 min, and 2% B at 45 min. Gradient elution was conducted in linear mode, and the separation was performed at 30 $^\circ\text{C}$. A post-column addition of 0.75 ml methanol/min was used.

LC Conditions for DNA Bases—LC/UV chromatography was conducted using gradient system 2 on a Hitachi L-7100 Pump equipped with Hitachi L-7400 UV detector (Hitachi, San Jose, CA). The separation employed a Phenomenex Synergi polar RP column (250 \times 4.6-mm inner diameter, 4 μm). Solvent A was 5 mM ammonium acetate in water, and solvent B was 5 mM ammonium acetate in acetonitrile. The flow rate was 1.0 ml/min, and the gradient was as follows: 6% B at 0 min, 6% B at 3 min, 20% B at 9 min, 20% B at 10 min, 80% B at 12 min, 80% B at 15 min, 6% B at 17 min, and 6% B at 25 min. Gradient elution was conducted in linear mode, and the separations were performed at ambient temperature. The amount of bases was calculated by interpolation from the calibration curves constructed with authentic bases.

LC Conditions for HedGuo Analysis—LC-APCI/MS analyses were conducted using gradient system 3 on Hitachi L-2200 Autosampler equipped with Hitachi L-2130 Pump (Hitachi, San Jose, CA). The separation employed a XTerra C18 column (250 \times 4.6-mm; inner diameter, 5 μm ; Waters). Solvent A was 5 mM ammonium acetate in water, and solvent B was 5 mM ammonium acetate in acetonitrile. The flow rate was 1.0 ml/min, and the gradient was as follows: 6% B at 0 min, 6% B at 3 min, 20% B at 9 min, 20% B at 13 min, 40% B at 22 min, 70% B at 27 min, 80% B at 28 min, 80% B at 32 min, 6% B at 34 min, and

5-Lipoxygenase-mediated Endogenous DNA Damage

6% B at 42 min. Gradient elution was conducted in linear mode, and the separations were performed at ambient temperature.

MS Conditions for Lipids Analysis—A TSQ Quantum Ultra AM triple quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with an APCI source was used in the ECAPCI negative ion mode. Operating conditions for the instrument was as follows: vaporizer temperature at 450 °C, heated capillary temperature at 250 °C, with the corona discharge needle set at 30 μ A. The sheath gas (nitrogen), auxiliary gas (nitrogen), and ion sweep gas (nitrogen) were 25, 3, and 3 (arbitrary units), respectively. Source collision-induced dissociation energy was 10 eV. Collision-induced dissociation was performed using argon as the collision gas in the radio frequency-only quadrupole. Targeted LC-ECAPCI/MRM/MS analysis was conducted on PFB derivatives. MRM transition for the following 16 lipids and 8 heavy isotope analog internal standards were monitored: 13(*R*)- and 13(*S*)-HODE-PFB, m/z 295 \rightarrow 195 (collision energy, 18 eV); 13(*S*)-[$^2\text{H}_4$]HODE-PFB, m/z 299 \rightarrow 198 (collision energy, 18 eV); 5(*R*)- and 5(*S*)-HETE-PFB, m/z 319 \rightarrow 115 (collision energy, 15 eV); 5(*S*)-[$^2\text{H}_8$]HETE-PFB, m/z 327 \rightarrow 116 (collision energy, 15 eV); 8(*R*)- and 8(*S*)-HETE-PFB, m/z 319 \rightarrow 155 (collision energy, 16 eV); 11(*R*)- and 11(*S*)-HETE-PFB, m/z 319 \rightarrow 167 (collision energy, 16 eV); 12(*R*)- and 12(*S*)-HETE-PFB, m/z 319 \rightarrow 179 (collision energy, 14 eV); 12(*S*)-[$^2\text{H}_8$]HETE-PFB, m/z 327 \rightarrow 184 (collision energy, 14 eV); 15(*R*)- and 15(*S*)-HETE-PFB, m/z 319 \rightarrow 219 (collision energy, 13 eV); 15(*S*)-[$^2\text{H}_8$]HETE-PFB, m/z 327 \rightarrow 226 (collision energy, 13 eV); LTB₄-PFB, m/z 335 \rightarrow 195 (collision energy, 18 eV); [$^2\text{H}_4$]LTB₄-PFB, m/z 339 \rightarrow 197 (collision energy, 18 eV); PGE₂-PFB, m/z 351 \rightarrow 271 (collision energy, 18 eV); [$^2\text{H}_4$]PGE₂-PFB, m/z 355 \rightarrow 275 (collision energy, 18 eV); PGD₂-PFB, m/z 351 \rightarrow 271 (collision energy, 18 eV); [$^2\text{H}_4$]PGD₂-PFB, m/z 355 \rightarrow 275 (collision energy, 18 eV); PGF_{2 α} -PFB, m/z 353 \rightarrow 309 (collision energy, 18 eV); [$^2\text{H}_4$]PGF_{2 α} -PFB, m/z 357 \rightarrow 313 (collision energy, 18 eV). Standard curves were constructed in the range of 0.20–200.00 pmol/10⁷ cells for 5(*S*)-HETE and LTB₄ and in the range of 0.02–20.00 pmol/10⁷ cells for the other compounds. Concentrations of lipids in the study samples were determined by interpolation from the standard curve regression lines.

MS Conditions for HedGuo Analysis—A TSQ Quantum Ultra AM instrument (Thermo Fisher Scientific) was operated used in the positive ion APCI mode. Operating conditions for the instrument was as follows: vaporizer temperature at 550 °C, heated capillary temperature at 180 °C, with the corona discharge needle set at 18 μ A. The sheath gas (nitrogen), auxiliary gas (nitrogen), and ion sweep gas (nitrogen) were 30, 3, and 0 (arbitrary units), respectively. Source collision-induced dissociation energy was –10 eV. Collision-induced dissociation was performed using argon as the collision gas in the radio frequency-only quadrupole. LC/APCI/MRM/MS analysis was conducted on PFB derivatives. The following MRM transitions were monitored: HedGuo-PFB, m/z 584 \rightarrow 468 (collision energy, 20 eV); $^{15}\text{N}_5$ -HedGuo-PFB, m/z 589 \rightarrow 473 (collision energy, 20 eV). A linear regression line was constructed in the range of 0.05–5.00 ng. Amounts of HedGuo in the DNA were determined by interpolation from regression line of and then

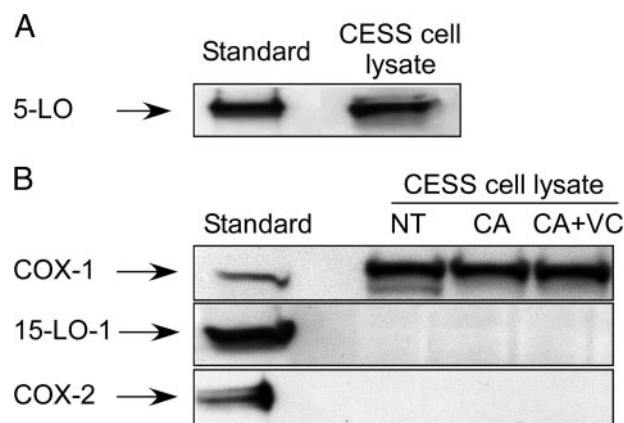


FIGURE 1. Western blot analyses of LO and COX in CESS cells. A, staining for 5-LO in CESS cells (cell lysate containing 50 μ g of protein). B, staining for COX-1, 15-LO-1, and COX-2 in CESS cells (cell lysate containing 50 μ g of protein) under experimental conditions. NT, no treatment; CA, treated with A23187 1.0 μ M; CA+VC, treated with A23187 1.0 μ M and vitamin C 1.0 mM.

converted to HedGuo-adducts/10⁷ normal bases from the DNA base analysis data.

RESULTS

In the following sections we present the result of a series of experiments designed to study the relationship between 5-LO-mediated lipid peroxidation and endogenous DNA damage. Western blot analysis was employed to profile the expression of lipid peroxidation enzymes in CESS cells. The cells were stimulated with calcium ionophore A23187 to activate the enzymes to generate lipid hydroperoxides, which were measured as reduced and secreted forms in the cell culture medium. MK886 as an inhibitor of FLAP, aspirin as an inhibitor of COX, or vitamin C as a mediator of lipid hydroperoxide decomposition was employed to elucidate the different pathways of lipid peroxidation. In all the cases, DNA was extracted from the cells to measure the endogenous DNA-adduct formation. Correlation of lipid peroxidation with DNA-adduct formation helped us to elucidate the role of specific enzymatic pathway in cellular DNA damage.

Expression of LOs and COXs in CESS Cells—CESS cells expressed 5-LO (Fig. 1A) and COX-1 (Fig. 1B) but not 15-LO-1 or COX-2 (Fig. 1B) as shown by Western blot analysis. Treatment with calcium ionophore A23187 alone or together with vitamin C for 24 h had no induction on expression of either 15-LO or COX-2 (Fig. 1B).

5-HETEs and LTB₄ Secreted from CESS Cells—5-HETE and LTB₄ are major lipid peroxidation products from 5-LO activity. Formation of 5-HETE and LTB₄ were measured to monitor the 5-LO activity in the cells treated with calcium ionophore A23187. Unstimulated CESS cells produced similar amounts of 5(*R*)-HETE (0.06 \pm 0.01 pmol/10⁷ cells) and 5(*S*)-HETE (0.07 \pm 0.01 pmol/10⁷ cells) (Figs. 2 and 3A). After stimulation with 1 μ M calcium ionophore A23187, 5(*S*)-HETE increased more than 3 orders of magnitude to 45.50 \pm 4.05 pmol/10⁷ cells, whereas the production of 5(*R*)-HETE only increased \sim 20-fold to 1.42 \pm 0.07 pmol/10⁷ cells (Figs. 3A and 4). LTB₄ secreted by unstimulated CESS cells was below the detection limit of the assay (Figs. 2 and 3B). The level of LTB₄ increased to 48.10 \pm 4.60 pmol/10⁷ cells (Figs. 3B and 4) with the treatment of calcium ionophore.

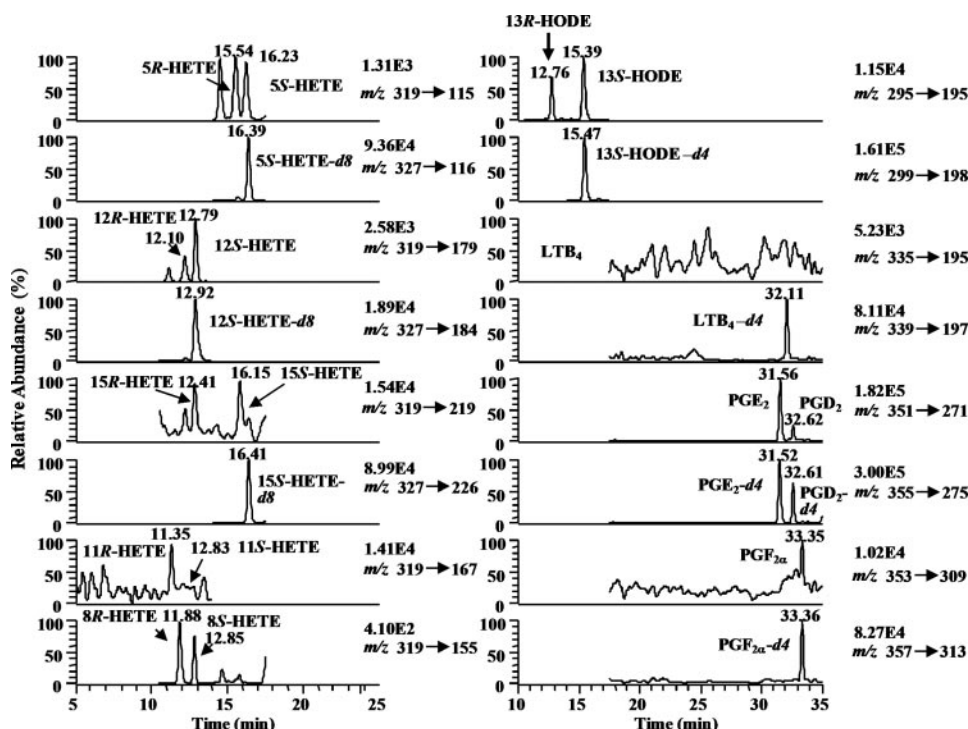


FIGURE 2. Chromatograms from targeted lipidomics analysis using LC-ECAPCI/MRM/MS for analysis of lipid metabolites from CESS cells. Chromatograms are shown for 5(*R,S*)-HETE (*m/z* 319 → *m/z* 115), 5(*S*-[²H₈])HETE (*m/z* 327 → *m/z* 116), 12(*R,S*)-HETE (*m/z* 319 → *m/z* 179), 12(*S*-[²H₈])HETE (*m/z* 327 → *m/z* 184), 15(*R,S*)-HETE (*m/z* 319 → *m/z* 219), 15(*S*-[²H₈])HETE (*m/z* 327 → *m/z* 226), 11(*R,S*)-HETE (*m/z* 319 → *m/z* 167), 8(*R,S*)-HETE (*m/z* 319 → *m/z* 155), 13(*R,S*)-HODE (*m/z* 295 → *m/z* 195), 13(*S*-[²H₄])HODE (*m/z* 299 → *m/z* 198), LTB₄ (*m/z* 335 → *m/z* 195), [²H₄]LTB₄ (*m/z* 339 → *m/z* 197), PGE₂ (*m/z* 351 → *m/z* 271), PGD₂ (*m/z* 351 → *m/z* 271), [²H₄]PGE₂ (*m/z* 355 → *m/z* 275), [²H₄]PGD₂ (*m/z* 355 → *m/z* 275), PGF_{2α} (*m/z* 353 → *m/z* 309), [²H₄]PGF_{2α} (*m/z* 357 → *m/z* 313).

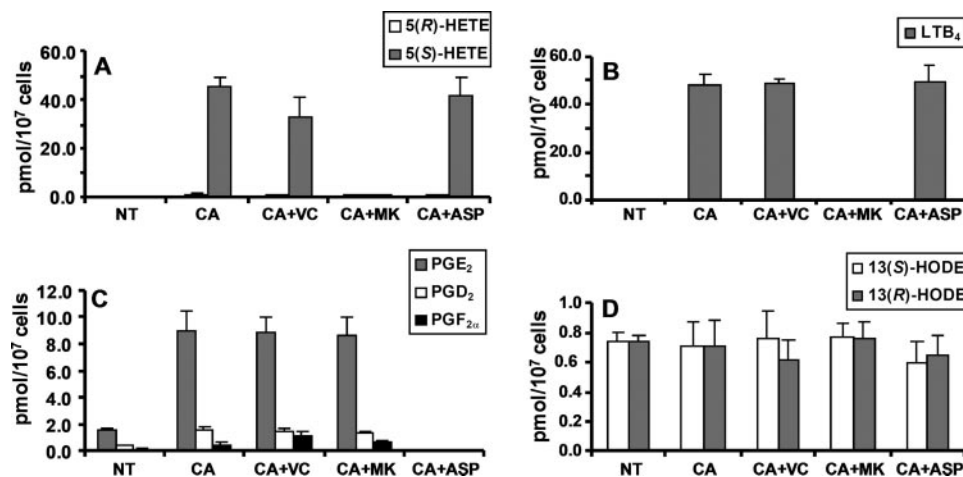


FIGURE 3. Amount of lipid peroxidation metabolites from CESS cells. A, 5-HETEs. B, LTB₄. C, PGE₂, PGD₂, and PGF_{2α}. D, 13-HODEs. NT, no treatment; CA, treated with 1.0 μM A23187; CA+VC, treated with 1.0 μM A23187 and 1.0 mM vitamin C; CA+MK, treated with 1.0 μM A23187 and 1.0 μM MK886; CA+ASP, treated with 1.0 μM A23187 and 200.0 μM aspirin. Analyses were performed by stable isotope dilution LC-ECAPCI/MRM/MS of PFB derivatives. Determinations were conducted in triplicate (means ± S.D.).

PGs Secreted from CESS Cells—PGs are the major lipid peroxidation products from COX activity. The formation of PGs was measured to reflect the COX activity in the cells. The levels of PGE₂, PGD₂, and PGF_{2α} for unstimulated cells were 1.57 ± 0.24, 0.47 ± 0.05, and 0.13 ± 0.03 pmol/10⁷ cells, respectively (Figs. 2 and 3C). Upon stimulation with 1 μM calcium ionophore A23187, PGE₂, PGD₂, and PGF_{2α} levels were increased

7-, 3-, and 4-fold to 9.10 ± 1.44, 1.55 ± 0.37, and 0.54 ± 0.16 pmol/10⁷ cells, respectively (Figs. 3C and 4).

13-HODEs Secreted from CESS Cells—13(*S*)-HODE is usually generated from linoleic acid by COX-2 or 15-LO activity. Basal levels of 13(*R*)- and 13(*S*)-HODE in unstimulated CESS cells were 0.78 ± 0.07 and 0.78 ± 0.05 pmol/10⁷ cells, respectively (Figs. 2 and 3D). Calcium ionophore A23187 did not stimulate the cells to produce 13(*R*)-HODE or 13(*S*)-HODE (Figs. 3D and 4).

8-, 11-, 12-, and 15-HETEs Secreted from CESS Cells—A variety of HETEs could be derived either from enzyme activities or nonenzymatically from ROS. The basal levels of 8(*R*)- and 8(*S*)-HETE in unstimulated CESS cells were 0.06 ± 0.03 and <0.01 pmol/10⁷ cells, respectively (Figs. 2 and 5A). Calcium ionophore A23187 stimulated the cells to produce 0.33 ± 0.13 pmol/10⁷ cells of 8(*R*)-HETE and 0.46 ± 0.12 pmol/10⁷ cells of 8(*S*)-HETE (Figs. 4 and 5A). The basal levels of 11(*R*)- and 11(*S*)-HETE in unstimulated CESS cells were 0.17 ± 0.06 and 0.29 ± 0.05 pmol/10⁷ cells, respectively (Figs. 2 and 5B). Calcium ionophore A23187 treatment increased the production of 11(*R*)-HETE ~5-fold to 0.82 ± 0.22 pmol/10⁷ cells with no effect on the production of 11(*S*)-HETE (Figs. 4 and 5B). Basal levels of 12(*R*)- and 12(*S*)-HETE in unstimulated CESS cells were 0.09 ± 0.05 and 0.13 ± 0.04 pmol/10⁷ cells, respectively (Figs. 2 and 5C). When the cells were stimulated with calcium ionophore, the amount of 12(*R*)-HETE was 0.70 ± 0.17 pmol/10⁷ cells, and the amount of 12(*S*)-HETE was similar at 0.75 ± 0.17 pmol/10⁷ cells (Figs. 4 and 5C). The basal levels of 15(*R*)- and 15(*S*)-

HETE in unstimulated CESS cells were 0.05 ± 0.01 and 0.16 ± 0.01 pmol/10⁷ cells, respectively (Figs. 2 and 5D). With calcium ionophore A23187 treatment, the production of 15(*R*)- and 15(*S*)-HETE increased to 1.31 ± 0.17 and 1.59 ± 0.28 pmol/10⁷ cells, respectively (Figs. 4 and 5D).

Effect of Vitamin C on Lipid Secretion by CESS Cells—Treatment of CESS cells with 1.0 mM vitamin C and 1 μM calcium

5-Lipoxygenase-mediated Endogenous DNA Damage

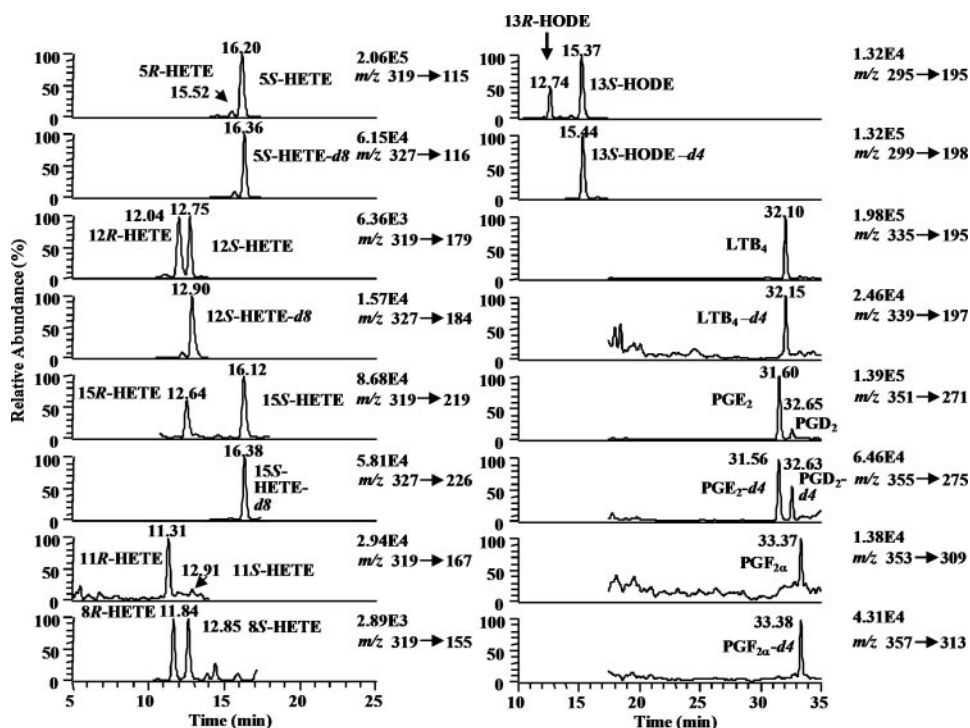


FIGURE 4. Chromatograms from targeted lipidomics analysis using LC-ECAPCI/MRM/MS for analysis of lipid metabolites from CESS cells treated with A23187 (1.0 μM). Chromatograms are shown for 5(*R,S*)-HETE (m/z 319 \rightarrow m/z 115), 5(*S*)-[$^2\text{H}_3$]HETE (m/z 327 \rightarrow m/z 116), 12(*R,S*)-HETE (m/z 319 \rightarrow m/z 179), 12(*S*)-[$^2\text{H}_3$]HETE (m/z 327 \rightarrow m/z 184), 15(*R,S*)-HETE (m/z 319 \rightarrow m/z 219), 15(*S*)-[$^2\text{H}_3$]HETE (m/z 327 \rightarrow m/z 226), 11(*R,S*)-HETE (m/z 319 \rightarrow m/z 167), 8(*R,S*)-HETE (m/z 319 \rightarrow m/z 155), 13(*R,S*)-HODE (m/z 299 \rightarrow m/z 198), 13(*S*)-[$^2\text{H}_4$]HODE (m/z 299 \rightarrow m/z 198), LTB₄ (m/z 335 \rightarrow m/z 195), [2H₄]LTB₄ (m/z 339 \rightarrow m/z 197), PGE₂ (m/z 351 \rightarrow m/z 271), PGD₂ (m/z 351 \rightarrow m/z 271), [$^2\text{H}_4$]PGE₂ (m/z 355 \rightarrow m/z 275), [$^2\text{H}_4$]PGD₂ (m/z 355 \rightarrow m/z 275), PGF_{2 α} (m/z 353 \rightarrow m/z 309), [$^2\text{H}_4$]PGF_{2 α} (m/z 357 \rightarrow m/z 313).

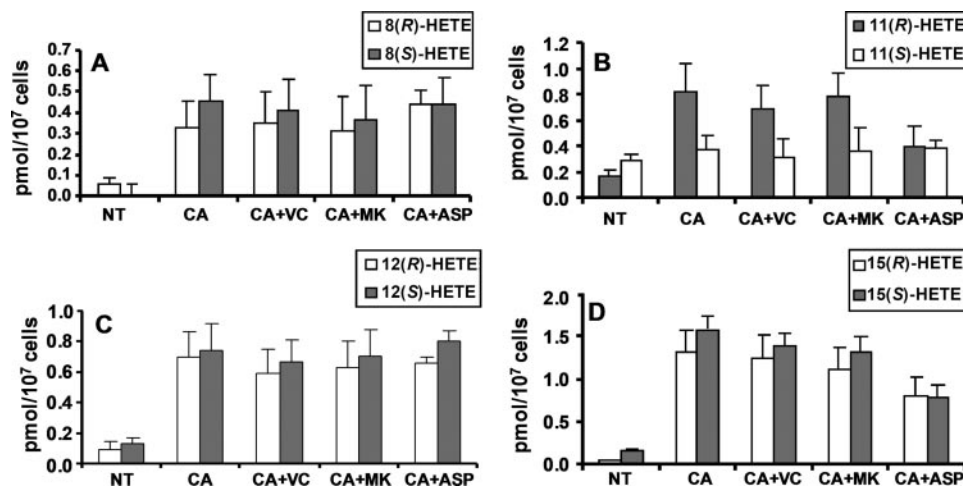


FIGURE 5. Amount of lipid peroxidation metabolites from CESS cells. A, 8-HETEs. B, 11-HETEs. C, 12-HETEs. D, 15-HETEs. NT, no treatment; CA, treated with 1.0 μM A23187; CA+VC, treated with 1.0 μM A23187 and 1.0 μM vitamin C; CA+MK, treated with 1.0 μM A23187 and 1.0 μM MK886; CA+ASP, treated with 1.0 μM A23187 and 200.0 μM aspirin. Analyses were performed by stable isotope dilution LC-ECAPCI/MRM/MS of PFB derivatives. Determinations were conducted in triplicate (means \pm S.D.).

ionophore A23187 resulted in a similar profile of lipids (Fig. 3). However, 5(*S*)-HETE (the major HETE) was decreased by 25% to 33.7 ± 6.5 pmol/ 10^7 cells (Fig. 3A). The amounts of LTB₄, PGs, and 13-HODEs (Figs. 3, B–D) were similar to cells treated with calcium ionophore A23187 alone. The amounts of other HETEs were also quite similar (Figs. 5, A–D).

Lipids Secreted from MK866-treated CESS Cells—The CESS cells were treated with FLAP inhibitor MK886 (29) at a con-

centration of 1.0 μM along with 1.0 μM calcium ionophore A23187. MK886 decreased the amount of secreted 5(*S*)-HETE to 1.20 ± 0.14 pmol/ 10^7 cells (Fig. 3A) and LTB₄ to 0.43 ± 0.07 pmol/ 10^7 cells (Fig. 3B). Production of 5(*R*)-HETE was decreased by 66% from 1.42 ± 0.07 to 0.47 ± 0.14 pmol/ 10^7 cells (Fig. 3A). The production of PGE₂, PGD₂, PGF_{2 α} , HODEs (Fig. 3, C and D), and other HETEs (Figs. 5, A–D) showed no significant changes compared with cells treated with calcium ionophore A23187 alone.

Lipids Secreted from Aspirin-treated CESS Cells—When the cells were treated with 200 μM aspirin in the presence of calcium ionophore A23187, the production of 5-HETEs and LTB₄ showed no significant difference from cells treated with calcium ionophore alone (Figs. 3, A and B). PGE₂, PGD₂, and PGF_{2 α} were reduced to 0.08 ± 0.02 , 0.02 ± 0.01 , and 0.08 ± 0.01 pmol/ 10^7 cells, respectively (Fig. 3C). 11(*R*)-HETE was decreased 50% to 0.40 ± 0.19 pmol/ 10^7 cells (Fig. 5B). 15(*R*)- and 15(*S*)-HETE were both decreased by 50% to 0.79 ± 0.23 and 0.80 ± 0.14 pmol/ 10^7 cells, respectively (Fig. 5D). 8-, 11(*S*)-, and 12-HETEs levels were similar to the cells treated with calcium ionophore A23187 alone (Figs. 5, A–C).

Analysis of HedGuo in CESS Cell DNA—HedGuo and its $^{15}\text{N}_5$ internal standard were converted to PFB-ester derivatives before analysis by stable isotope dilution LC-APCI/MRM/MS (Fig. 6A). The basal level of HedGuo in CESS cells was 2.41 ± 0.35 adducts/ 10^7 normal bases (Figs. 6B and 7). When treated with calcium ionophore for 24 h, the DNA-adduct level increased to 6.31 ± 0.73 adducts/ 10^7 normal bases (Figs. 6C and 7). Vitamin C, in the presence of calcium ionophore, further increased the HedGuo-adduct formation by 16% to 7.33 ± 0.60 adducts/ 10^7 normal bases (Figs. 6D and 7).

Analysis of HedGuo from CESS Cells Treated with MK886 or Aspirin—The amount of HedGuo in CESS cells stimulated with calcium ionophore A23187 in the presence of MK886 was decreased to base-line levels of 2.92 ± 0.50 adducts/ 10^7 normal bases (Figs. 6E and 7). There were 6.35 ± 0.57 adducts/ 10^7

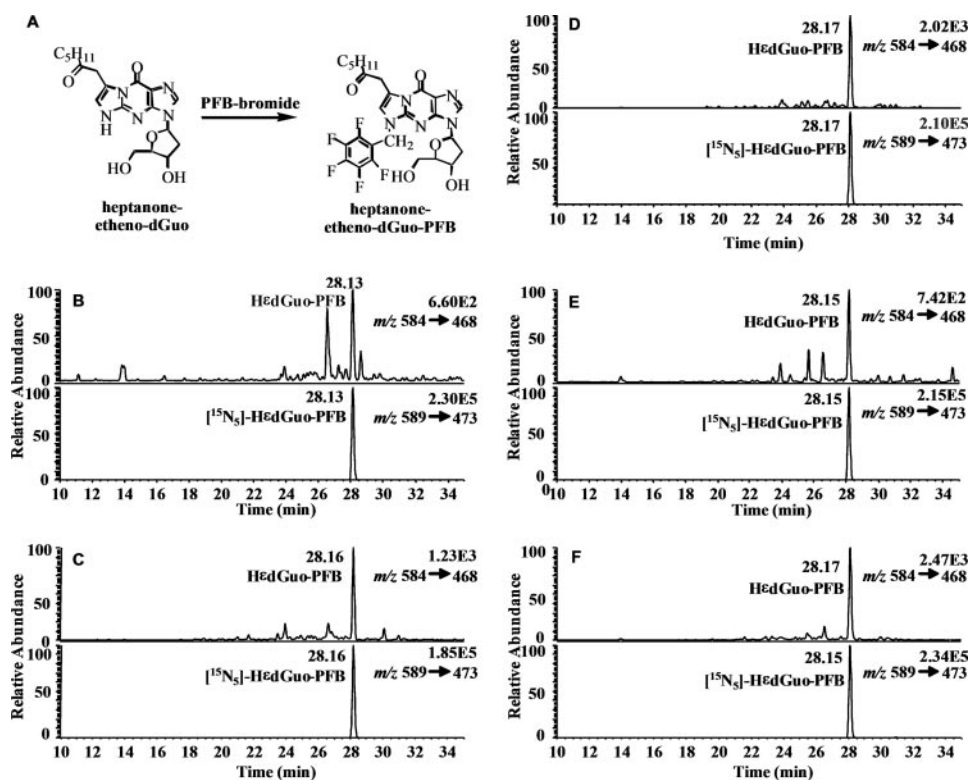


FIGURE 6. Representative of LC-APCI/MRM/MS chromatograms of HedGuo-PFB (m/z 584 \rightarrow m/z 468) and $^{15}\text{N}_5$ -HedGuo-PFB (m/z 589 \rightarrow m/z 473). A, scheme of derivatization of HedGuo-PFB. B, chromatograms from unstimulated CESS cells. C, chromatograms from CESS cells treated with $1.0\ \mu\text{M}$ A23187. D, chromatograms from CESS cells treated with $1.0\ \mu\text{M}$ A23187 and $1.0\ \text{mM}$ vitamin C. E, chromatograms from CESS cells treated with $1.0\ \mu\text{M}$ A23187 and $1.0\ \mu\text{M}$ MK886. F, chromatograms from CESS cells treated with $1.0\ \mu\text{M}$ A23187 and $200.0\ \mu\text{M}$ aspirin.

normal bases in the cells treated with calcium ionophore A23187 and aspirin (Figs. 6F and 7).

DISCUSSION

Leukocytes and lymphocytes are known to express COX-1, COX-2, 5-LO, and 15-LO-1 (35–37), which are all capable of generating arachidonic acid-derived lipid hydroperoxides (1, 32, 38). Western blot analyses revealed that the lymphoblastoid CESS cell line did not express COX-2 or 15-LO-1. Furthermore, COX-2 and 15-LO-1 were not induced by the various treatments used in the present study. This excluded the possibility that lipid peroxidation had originated from these two enzymes. However, both 5-LO and COX-1 were constitutively expressed in the CESS cells (Fig. 1).

Products of the 5-LO pathway have been identified as major arachidonic acid metabolites in many cell types (12, 15, 17, 23). CESS, a human lymphoblastic cell line has been reported to express both 5-LO and FLAP mRNA. In addition, 5-HETE was identified as one of the major arachidonic acid metabolites formed in CESS cells upon stimulation with calcium ionophore (33, 34). This made the CESS cells an ideal model to examine the effects of lipid hydroperoxide-derived DNA damage. No lipid profiling has been conducted in CESS cells to determine the relative contribution of different enzymatic and nonenzymatic pathways to lipid peroxidation. Therefore, a targeted chiral lipidomics approach was used to examine the endogenously produced lipid peroxidation products secreted from

the cells into the culture medium (39, 40). There was a substantial and stereoselective secretion of 5(S)-HETE upon stimulation of the CESS cells with calcium ionophore A23187. This confirmed the presence of a functional 5-LO activity (Fig. 3A). The concomitant secretion of substantial amounts of LTB_4 provided additional confirmation of the 5-LO activity (Fig. 3B). Interestingly, almost equimolar amounts of 5(S)-HETE and LTB_4 were generated. This suggested that sufficient 5(S)-HpETE could have been generated to undergo homolytic decomposition to ONE. MK886, a FLAP inhibitor, inhibited 97% of 5(S)-HETE production and 99% of LTB_4 production (Fig. 3, A and B). This meant that it was possible to determine whether any ONE-derived DNA adducts arose from 5-LO-mediated lipid hydroperoxide formation.

In keeping with the identification of COX-1 expression in the CESS cells (Fig. 1), low levels of PGs (Fig. 3C) together with 11(R)-HETE (Fig. 5B), 15(S)-HETE, and 15(R)-HETE (Fig. 5D) were observed. COX-1 can convert arachidonic acid into PGs, 11(R)-HETE, 15(R)-HETE, and 15(S)-HETE. The slight enantioselectivity for formation of 15(S)-HETE observed in the present study is consistent with previous findings for COX-1 (38). With aspirin treatment, 11(R)-HETE was reduced to the level of 11(S)-HETE. Meanwhile, 15(R)- and 15(S)-HETE were both decreased so that the 15-HETEs became racemic. The 11-HETEs (Fig. 5B) and 15-HETEs (Fig. 5D) observed in the presence of aspirin are thought to arise from ROS-mediated oxidation of arachidonic acid. The small amounts of racemic 8-HETEs (Fig. 5A) and 12-HETEs (Fig. 5C) are also thought to arise from ROS-mediated oxidation of arachidonic acid. The reduction of 5(S)-HpETE to 5(S)-HETE requires GSH as a co-factor (41). This suggests that the substantial amounts of 5(S)-HpETE formed after calcium ionophore A23187 stimulation in the CESS cells (Fig. 3A) might have compromised intracellular levels of GSH. This would result in oxidative stress (1), the induction of ROS-mediated lipid peroxidation, and the formation of racemic HETEs.

The 5-LO-derived arachidonic acid metabolites 5(S)-HETE and LTB_4 were shown to be the predominant eicosanoids, produced by the CESS cells at levels for each of $\sim 50\ \text{pmol}/10^7$ cells (Fig. 3, A and B). They were followed by PGE_2 , PGD_2 , and $\text{PGF}_{2\alpha}$ for a total of $\sim 11\ \text{pmol}/10^7$ cells, which was an order of magnitude below the 5-LO-derived metabolites (Fig. 3C). Non-enzymatically produced 8-, 11-, 12-, and 15-HETEs were more than an order of magnitude lower than 5(S)-HETE at a total of

5-Lipoxygenase-mediated Endogenous DNA Damage

~4 pmol/10⁷ cells (Fig. 5, A–D). Biosynthesis of 15-HETEs and 11-HETE by COX-1 was 25-fold lower than 5(S)-HETE at ~2 pmol/10⁷ cells (Fig. 5, B and D). Therefore, 5-LO-derived 5(S)-HpETE was by far the primary lipid peroxidation product formed in CESS cells. ROS- and COX-1-mediated arachidonic acid oxidation contributed only minor amounts of lipid peroxidation products.

Three DNA adducts were detected in the *in vitro* reaction between 5(S)-HpETE and dGuo (Scheme 1). Two of the DNA adducts (edGuo and HedGuo) were also detected in the DNA of CESS cells. However, the 5,8-dioxo-6-octenoic acid-derived CPedGuo (a carboxylate-containing DNA-adduct) was not detected (Scheme 1). The stable isotope dilution LC-APCI/MS methodology used for the analysis of CPedGuo and HedGuo had similar detection limits. Therefore, the inability to detect CPedGuo formation was not because of analytical problems. Instead, we consider that the lack of CPedGuo is because of the inability of 5,8-dioxo-6-octenoic acid to translocate across the nuclear membrane and react with DNA. In previous studies we showed that the related carboxylate-containing bifunctional electrophile 9,12-dioxo-10-dodecenoic acid could not translocate across the plasma membrane (28). In contrast, the corresponding methyl ester derivative readily crossed the plasma membrane where it was converted to free 9,12-dioxo-10-dodecenoic acid by cytosolic esterases. Previous studies have shown that 5-LO is localized in the cytosol and that it translocates to both inner and outer nuclear membranes upon activation (16). This suggests that the 5(S)-HpETE formed on the outer membrane underwent homolytic decomposition to ONE and 5,8-dioxo-6-octenoic acid as well as undergoing reduction to 5(S)-HETE or conversion to LTB₄. In contrast, it appears that 5(S)-HpETE formed on the inner leaflet of the nuclear membrane was reduced more efficiently to 5(S)-HETE and/or was converted more efficiently to LTB₄ (Scheme 1).

HedGuo was quantified in the CESS cell DNA because it is a specific product of lipid peroxidation, whereas edGuo can arise from other pathways (38). Calcium ionophore A23187 stimulation of the CESS cells resulted in a 3-fold increase in HedGuo over basal levels (Fig. 7). MK886, which inhibits FLAP (42), decreased the production of 5(S)-HETE by 97% (Fig. 3A) and HedGuo by almost 90% to a level that was not significantly different from the basal levels (Fig. 7). In addition, aspirin (a nonspecific COX inhibitor) had no detectable effect on HedGuo formation. Aspirin was shown previously to reduce HedGuo formation to basal levels in epithelial cells that stably expressed COX-2 (38). This confirmed that HedGuo formation in CESS cell DNA arose primarily from 5-LO-mediated lipid peroxidation.

Vitamin C was shown to induce homolytic decomposition of lipid hydroperoxides into bifunctional electrophiles *in vitro*, which suggested that it would cause increased DNA adduct formation *in vivo* (27). Subsequently, it was shown that vitamin C increased 15-HpETE-mediated HedGuo formation in cells that stably expressed COX-2 (38). In the present study, vitamin C increased HedGuo formation in 5-LO-expressing CESS cells (Fig. 7). These results provide additional data to support the concept that vitamin C can have opposing effects on DNA damage. It can scavenge ROS and prevent oxidative DNA damage

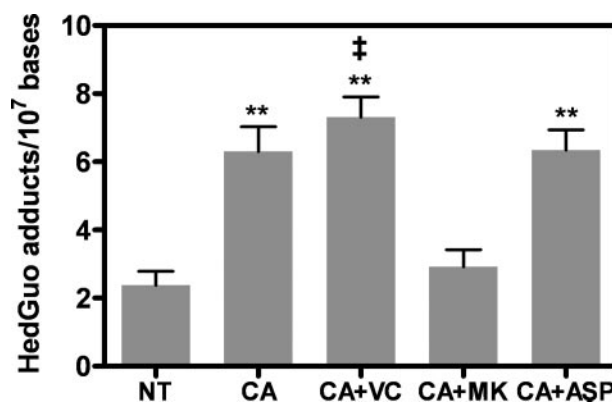


FIGURE 7. Amount of HedGuo in CESS cells. NT, no treatment; CA, treated with 1.0 μ M A23187; CA+VC, treated with 1.0 μ M A23187 and 1.0 mM vitamin C; CA+MK, treated with 1.0 μ M A23187 and 1.0 μ M MK886; CA+ASP, treated with 1.0 μ M A23187 and 200.0 μ M aspirin. Analyses were performed by stable isotope dilution LC-APCI/MRM/MS of PFB derivatives. Determinations were conducted in triplicate (means \pm S.D.). **, $p < 0.001$ versus untreated cells; †, $p < 0.05$ versus cells treated with A23187 alone (Student's *t* test).

(43–45), whereas it can stimulate an increase in lipid hydroperoxide-mediated damage.

5-LO activity has been linked to the underlying mechanisms of inflammatory diseases (46–48). There is increasing evidence of a link between inflammation, oxidative stress, and the progression of both cancer and atherosclerosis (49, 50). Increased oxidative DNA damage (as determined by 8-oxo-dGuo measurements) has been observed in cancer and cardiovascular disease (51–55). We showed previously that lipid hydroperoxide-mediated DNA damage might also be an important mediator of cancer and cardiovascular disease (38). The current study has provided additional support for this concept. Lipid hydroperoxide-derived modifications to DNA are highly mutagenic (56), which provides a link to the role of this pathway in carcinogenesis. Previous studies have also implicated the 5-LO pathway in carcinogenesis. 5-LO is expressed in multiple cancer cells (24) and overexpressed in human prostate and pancreatic cancer tissue (24–26, 57). This has led to the suggestion that 5-LO and FLAP inhibitors might be useful as chemoprevention agents (58–60).

In summary, our targeted chiral lipidomics approach has provided a powerful tool to study pathways of cellular COX-1-, 5-LO-, and ROS-mediated lipid peroxidation and to examine their relationship to cellular DNA damage. Previous studies have suggested that 5-LO-derived PUFA metabolites are potential mediators of carcinogenesis (61, 62). Our data suggest that 5-LO-mediated DNA damage could also be important.

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