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15-HYDROXYEICOSATETRAENOIC ACID INHIBITS SUPEROXIDE ANION GENERATION BY HUMAN NEUTROPHILS: RELATIONSHIP TO LIPOXIN PRODUCTION

CHARLES N. SERHAN* and EILEEN REARDON

Hematology Division, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, 75 Francis Street, Boston, MA 02115

Human neutrophils can aggregate, degranulate, and release mediators of inflammation including oxygen radicals and lipoxygenase (LO)-derived products of arachidonic acid. The regulation of 5- and 15-lipoxygenases appears to be important since their products (e.g. leukotrienes and lipoxins) display unique spectra of bioactions. Addition of 15-HETE, a product of the 15-LO, to neutrophils in suspension dramatically shifted the LO products generated and led to a dose-dependent increase in lipoxins, while the production of leukotriene B_4 and its ω-oxidation products (i.e. 20-COOH-LTB_4 and 20-OH-LTB_4) was inhibited. Exogenous 15-HETE also dose-dependently inhibited the generation of superoxide anions induced by either the chemotactic peptide f-met-leu-phe or the divalent cation ionophore A23187. Neither lipoxin A_4 nor lipoxin B_4 (10^{-8}–10^{-6} M) inhibited O_2^- generation induced by either f-met-leu-phe or A23187. These results indicate that in addition to serving as a substrate for lipoxin generation, 15-HETE also inhibits superoxide anion generation by human neutrophils. Together they provide further evidence to suggest that products of the 15-lipoxygenase may serve a regulatory role at inflammatory loci.

KEY WORDS: Lipoxygenase products, human neutrophils, O_2^- generation.

ABBREVIATIONS: 15-HETE, 15S-hydroxy-5,8,11-cis-13-trans-eicosatetraenoic acid; RP-HPLC, reverse phase high pressure liquid chromatography; lipoxin A_4 (LXA_4), 5S, 6R, 15S-trihydroxy-7,9,13-trans-11-cis-eicosatetraenoic acid; lipoxin B_4 (LXB_4), 5S,14R,15S-trihydroxy-6,10,12-trans-8-cis-eicosatetraenoic acid; LTB_4, leukotriene B_4, 5S,12R-dihydroxy-6,14-cis-8,11-trans-dihydroxyeicosatetraenoic acid; 5-HETE, 5S-hydroxy-8,11,14-cis-6-trans-eicosatetraenoic acid.

INTRODUCTION

Upon activation, human neutrophils aggregate, generate active oxygen species, and release arachidonic acid which can be oxygenated by lipoxygenases.[1] While the 5-lipoxygenase (LO) is key in the formation of leukotrienes, another major route of arachidonic acid metabolism is initiated by the 15-LO.[2] A product of this enzyme, namely 15-HETE, has been identified in a variety of tissues including lung,[3] tracheal epithelial cells,[4] and both normal and atherosclerotic vessels.[5] In addition, following antigenic challenge, bronchial lavage fluids from allergic subjects contain high levels of 15-HETE.[6] Therefore, the actions and metabolic fate of this product are of interest.

Interactions between the 5- and 15-lipoxygenase pathways lead to the formation of lipoxins.[1,2] Products of this series display bioactivities distinguishable from those of either leukotrienes or prostaglandins.[2] In this report, evidence is presented indicating

* Author for correspondence.

that 15-HETE, which is transformed to lipoxins by human neutrophils, can also inhibit superoxide anion generation by these cells.

MATERIALS AND METHODS

HPLC solvents were obtained from American Scientific Products, Burdick and Jackson, Muskegon, MI. Synthetic lipoxin A_4, lipoxin B_4, LTB_4 and other eicosanoids used as chromatographic standards were from Biomol Research Laboratories, Inc. (Philadelphia, PA). 15S-HPETE was prepared by incubation of arachidonic acid (Nuchek Prep) with soybean lipoxygenase and 15S-HETE was prepared by $SnCl_2$ reduction of 15S-HPETE.[7]

Cell suspensions and incubation conditions

Neutrophils were isolated from heparinized blood, just after venipuncture, by dextran sedimentation followed by Ficoll-Hypaque gradient centrifugation. Contaminating red blood cells were removed by hypotonic lysis followed by centrifugation.[8] The cells were suspended in Dulbecco's phosphate-buffer saline containing both $CaCl_2$ (0.6 mM) and $MgCl_2$ (1.0 mM), pH 7.4 and warmed 5 min at 37°C prior to additions (vide infra). These suspensions contained 98 $\pm$ 1% neutrophils as determined by light microscopy. The integrity of cells in suspension was monitored both before and during incubation conditions by determining their ability to exclude trypan blue. Under all experimental conditions reported, $< 3\%$ of the cells in suspension were permeable to trypan blue.

In studies with lipoxygenase-derived products, neutrophils ($\sim 30 \times 10^6$ cells/ml; 1 ml each incubation) were exposed to either the ionophore A23187 alone or the ionophore plus 15-HETE (see Results) added in ethanol (the final EtOH concentration did not exceed 0.1% vol/vol). For studies in which O_2^- generation was monitored, PMN (5×10^6 cells/1 ml) were incubated (37°C) in the presence or absence of cytochalasin B (3 min) followed by addition of stimuli. The generation of superoxide anion was determined by superoxide dismutase inhibitable reduction of ferricytochrome C.[9]

Analysis of lipoxygenase-derived products

Products were extracted and analyzed on a RP-HPLC system equipped with an Altex Ultrasphere-ODS column (4.6 mm $\times$ 25 cm) and a photodiode array spectral detector linked to an AT&T PC6300. Post-run analyses were performed with a Nelson Analytical 3000 series chromatography data system (Paramus, NJ) and Wavescan 2140-202 (Bromma, Sweden) software as described.[10]

RESULTS

Neutrophils exposed to A23187 generate leukotriene B_4 as well as lipoxins from endogenous sources of arachidonic acid where the amounts of leukotrienes are greater than those of lipoxins.[10] They can also transform 15-HPETE to lipoxins. In this case, exposure to exogenous 15-HPETE led to both activation of a 5-lipoxygenase activity,

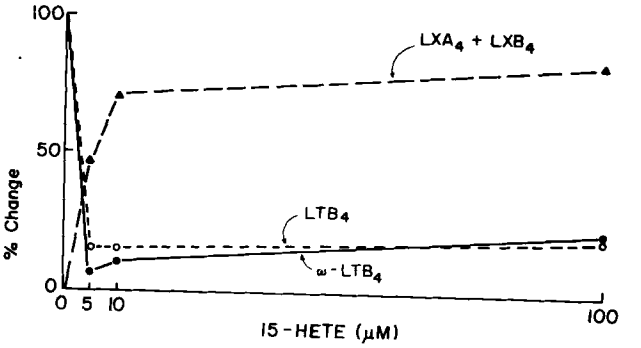

FIGURE 1 Relationship between leukotriene and lipoxin production. Neutrophils (30×10^6) were incubated with A23187 (2.5 μM) in the presence and absence of varying amounts of exogenous 15-HETE for 20 min at 37°C. Incubations were terminated by addition of methanol and products were extracted and analyzed by RP-HPLC (see Methods). In the absence of 15-HETE, A23187 stimulated the formation of lipoxygenase products from endogenous sources of arachidonate. The values were 316.1 ± 136.6 ng (LTB_4), 94.2 ± 17.6 ng (20-COOH-LTB_4), 6.4 ± 3.5 ng (LXA_4), and 1.1 ± 0.5 ng (LXB_4) as determined in ref. 10. Data are presented as the percent change in products following addition of 15-HETE (n = 3).

and to the consumption of this eicosanoid for the formation of lipoxins. 15-HETE is also transformed to lipoxins[1] and, in previous studies, 15-HETE has been shown to inhibit leukotriene B_4 production.[11,12] Since the balance between lipoxygenase-derived products may be of importance in both pathophysiological and physiological circumstances, we recently examined the relationship between leukotriene and lipoxin formation by human neutrophils.[10] Addition of 15-HETE to A23187-stimulated neutrophils led to a dose-dependent increment in the formation of lipoxins. In the same incubations, 15-HETE inhibited the production of both LTB_4 and its ω-oxidation product 20-COOH-LTB_4 (Figure 1; see ref.[10] for resolution of products). Results from time course studies with radiolabeled 15-HETE revealed that both inhibition of leukotriene formation and the conversion of 15-HETE to lipoxins are rapid events evident within 15 seconds of its addition to neutrophils in suspension.[10]

Effect of 15-HETE on O_2^- generation

Results of recent studies indicate that exogenous 15-HETE inhibits both Ca^{2+} mobilization and degranulation induced by activated human neutrophils.[13] Since we observed that 15-HETE was not biologically stable, in that it was rapidly (< 15 s) transformed by these cells,[10] the effect of 15-HETE was examined on another neutrophil response (i.e. O_2^- generation). 15-HETE inhibited A23187-induced O_2^- generation both in the presence and absence of cytochalasin B. A similar trend was observed when the chemotactic peptide f-met-leu-phe was the agonist. In both cases the inhibitory action of 15-HETE was more pronounced when the cells were incubated with cytochalasin B (Figure 2). Although the inhibitory action of 15-HETE was in each case clearly dose-dependent, the response curves were not sigmoidal. In two separate experiments, neither LXA_4 nor LXB_4 (10^{-8}–10^{-6} M) inhibited O_2^- generation induced by either f-met-leu-phe (10^{-7} M) or A23187 (2.5 μM).

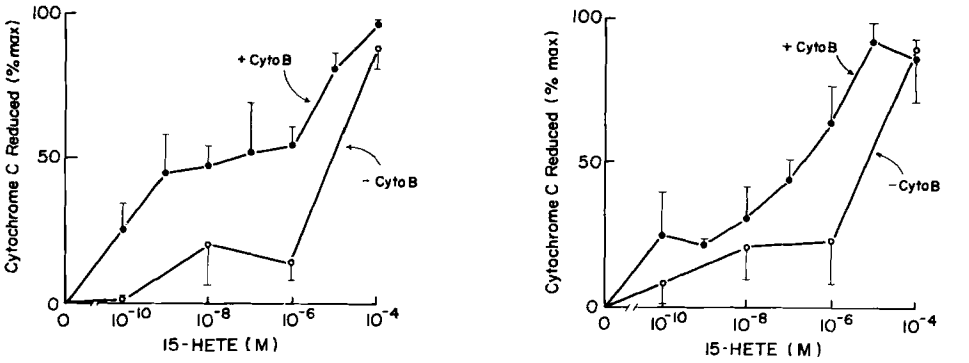

FIGURE 2 Superoxide generation by human neutrophils: inhibitory effect of 15-HETE. Neutrophils (5×10^6) were incubated for 3 min at 37°C in the presence or absence of cytochalasin B (10 μg/ml) in PBS (1 ml, pH 7.45). 15-HETE, at the indicated concentrations, was added at time zero together with either f-met-leu-phe (10^{-7} M) or A23187 (2.5 μM) and the incubations continued for 10 min at 37°C. Cell-free supernatants were obtained by rapid centrifugation and assayed for SOD-inhibitable reduction of cytochrome C. Results are presented as the percent inhibition of maximum reduction of cytochrome C generated with A23187 (2.5 μM) $\pm$ cyto B (left panel) and FMLP (10^{-7} M) $\pm$ cyto B (right panel) with the indicated concentrations of 15-HETE. In the absence of 15-HETE, neutrophils incubated with cytochalasin B and A23187 (2.5 μM) reduced 43 $\pm$ 8 nmoles of cytochrome C/mg of protein, and with cytochalasin B and FMLP (10^{-7} M) reduced 65 $\pm$ 10 nmoles of cytochrome C/mg of protein. These data represent averages of duplicate determinations from individual donors (stimulated cells minus appropriate vehicle controls) of 3 or more separate experiments (mean $\pm$ S.E.M.).

DISCUSSION

Previous results indicate that, when added to human neutrophils, 15-HETE inhibits Ca^{2+} mobilization, degranulation, and LTB_4 formation.[13] 15-HETE is also transformed to lipoxins by activated neutrophils,[1,2] and evidence for a relationship between leukotriene and lipoxin production has been presented in these cells (Figure 1 and ref.[10]). Results of these experiments indicate that 15-HETE also inhibits the generation of superoxide anions induced by either the chemotactic peptide f-met-leu-phe or the ionophore A23187 (Figure 2).

In contrast to the actions of 15-HETE, recent results indicate that 5-HETE stimulates neutrophils to mobilize Ca^{2+}, translocate protein kinase C, generate superoxide anions and release lysosomal enzymes.[9,14] When added to human neutrophils, LXA_4 stimulates migration at 1 nM and exhibits a variable effect as an inducer of chemiluminescence at concentrations $\leqslant 1\ \mu$M.[15] In the present study, neither LXA_4 nor LXB_4 (10^{-8}–10^{-6} M) inhibited O_2^- generation induced by either f-met-leu-phe or A23187. Taken together the finding that 15-HETE is transformed to lipoxins and inhibits both the generation of leukotrienes and superoxide anions by human neutrophils provides further evidence to suggest that the formation of 15-HETE may be an important event in inflammation and cell injury.

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