NOVEL STRUCTURAL DETERMINANTS OF THE HUMAN NEUTROPHIL CHEMOTACTIC ACTIVITY OF LEUKOTRIENE B*

BY EDWARD J. GOETZL AND WALTER C. PICKETT

From the Howard Hughes Medical Institute Laboratory at Harvard Medical School; the Departments of Medicine, Harvard Medical School and the Brigham and Women's Hospital, Boston, Massachusetts 02115; and the Department of Pharmacology, University of Colorado School of Medicine, Denver, Colorado 80262

Leukotrienes are a unique class of potent humoral and cellular mediators that are generated by the specific lipoxygenation and subsequent enzymatic conversions of arachidonic acid in basophils, mastocytoma cells, polymorphonuclear leukocytes (PMN) and mononuclear leukocytes, and human lung tissue (1–6). The structural characteristics shared by the leukotriene mediators include three conjugated double bonds, a 5-hydroxy group, and either a 6-sulfido-cysteine(yl) or 12-hydroxy group. 5-hydroxy-6-sulfido-glutathionyl-eicosa-7,9,11,14-tetraenoic acid (leukotriene C₄ [or LTC₄]), where the subscript 4 indicates the total number of double bonds) and 5-hydroxy-6-sulfido-cysteiny1-glycine-7,9,11,14-tetraenoic acid (LTD₄), which are the principal active constituents of the slow-reacting substance of anaphylaxis (termed SRS-A), constrict pulmonary airways with a potency up to 20,000 times greater than that of histamine and alter the tone and permeability of the microvasculature (7). 5(S),12(R)-di-hydroxy-eicosa-6,8,10(trans/trans/cis),14(cis)-tetraenoic acid, which is designated LTB₄ (6), is chemotactic and chemokinetic for human and rat PMN and, to a lesser extent, mononuclear leukocytes (8–10). The maximal human neutrophil chemotactic response elicited by LTB₄ is similar in magnitude to those evoked by synthetic formyl-methionyl peptides or fragments of the fifth component of human complement, and the neutrophil chemotactic potency of LTB₄ is far greater than that of the mono-hydroxy-eicosatetraenoic acids (HETE) derived from arachidonic acid, whereas neither LTC₄ nor platelet-derived tri-HETE exhibit leukocyte chemotactic activity (8). The results of our study indicate that the conformation of the hydroxyl-groups and the geometry of the double bonds in the conjugated triene are the critical structural determinants of the potency of LTB₄ as a neutrophil chemotactic factor.

Materials and Methods

Preparation and Purification of Lipoxygenase Products of Arachidonic Acid. Neutrophils that had been isolated from venous blood of normal subjects were incubated for 30 min at 37°C at a concentration of 5 × 10⁷ neutrophils/ml in Hanks' balanced salt solution containing 0.02 M Tris-HCl (pH 8), 10 μM indomethacin, 5 μM calcium ionophore A23187, and 0.5 mg/ml of arachidonic acid. The HETE were extracted and resolved from both the residual arachidonic acid and more polar products by silicic acid column chromatography (6, 8). LTB₄ and 5-HETE

* Supported in part by grants HL 25785 and HL 19777 from the National Institutes of Health.
were purified by reverse-phase high performance liquid chromatography (HPLC) on a C18 ODS column (Ultrasphere; Alliance Scientific, Inc., Berkeley, Calif.) that was developed isocratically at a flow rate of 1 ml/min with methanol:water:glacial acetic acid (78:22:0.01, vol:vol:vol). The 5,12-di-HETE and 5,6-di-HETE isomers contained in the eluates of the silicic acid column were resolved from LTB4 and mce-HETE and were partially purified by reverse-phase HPLC on Nucleosil C-18, 5 \( \mu \)m (Mauchery-Nagel, Düren, Federal Republic of Germany) for 60 min. The 5,12-di-HETE and the 3,6-di-HETE were purified by rechromatography separately on the Nucleosil column utilizing methanol:water:glacial acetic acid (65:35:0.01, vol:vol:vol) and methanol:water:glacial acetic acid (75:25:0.01, vol:vol:vol), respectively. The highly purified LTB4 and di-HETE isomers contained <1% total of the other isomers.

The chromatographic characteristics of the two 5,6-di-HETE stereoisomers, for which the conformation of the hydroxyl-groups and the geometry of the double bonds have not been determined, and the two 5,12-all-trans-di-HETE, have been described previously (6). 5,6-di-HETE-a eluted before 5,6-di-HETE-b from the second Nucleosil C-18 column. The 5,12-di-HETE were converted into dimethoxy carbonyl derivatives, subjected to oxidative ozonolysis, and treated with diazo-methane, and the products were analyzed by gas chromatography-mass spectrometry to determine the configuration of the 5- and 12-hydroxy-groups (6).

LTB4 elicited a neutrophil chemotactic response that was detectable at a concentration of 0.3 ng/ml or \( \sim 10^{-9} \) M and was maximal at 30 ng/ml or \( \sim 10^{-7} \) M (Fig. 1). The maximal neutrophil chemotactic response to LTB4 was similar in magnitude to those evoked by the chemotactic fragments of C5 (56.6 ± 7.4, mean ± SD, \( n = 4 \)) or by \( 10^{-8} \) M formyl-methionyl-leucyl-phenylalanine (f-Met-Leu-Phe) (60.3 ± 9.2). The other di-HETE examined were significantly less potent than LTB4 as chemotactic stimuli, but at a concentration of 1,000 ng/ml evoked maximal chemotactic responses that were not significantly different from that seen with LTB4 at 10 ng/ml. Thus LTB4 was \( \sim 100 \)-fold more potent than the other di-HETE, when comparing the concentrations required to stimulate maximal chemotactic responses. An analysis of the concentrations needed to achieve a response equal to 50% of the maximal level indicated that the ratio of potency of LTB4 to the analogues ranged from 15 for stereoisomer a of the 5,6-di-HETE to >100 for 5(S),12(S)-all-trans-di-HETE. The
Derivatives of LTB4
- LTB4
- Acetyl-LTB4
- 5(S), 12(R)-all-trans-di-HETE
- 5(S), 12(S)-all-trans-di-HETE
- 5,6-di-HETE
- 5,6-dio-LTB4
- Non-reactive

Analogues of LTB4
- LTB4
- 5(S), 12(R)-all-trans-di-HETE
- 5(S), 12(S)-all-trans-di-HETE
- 5,6-di-HETE
- 5,6-dio-LTB4
- Non-reactive

Fig. 1. Human neutrophil chemotactic activity of derivatives and analogues of leukotriene B4. The points and brackets represent the mean ± SD of the results of eight determinations of activity for LTB4, five for acetyl-LTB4 and four for the other factors. The designation “all-trans” refers to the geometry of only the conjugated double bonds in the triene portion of the molecule. LTB4 was significantly more chemotactic for neutrophils (P < 0.01) than was acetyl-LTB4 at concentrations of 0.3–10 ng/ml, 5,6-di-HETE-a at 0.3–30 ng/ml, and the other analogues at 0.3–100 ng/ml.

5(S), 12(R)-all-trans-di-HETE, in which the 5- and 12-hydroxyl groups possess a different conformation, was substantially more potent than the isomer with optically equivalent 5(S)- and 12(S)-hydroxyl substituents.

Acetylation of LTB4 decreased the chemotactic activity significantly at concentrations of 0.3–10 ng/ml, but not at maximally active concentrations, while incubation in pyridine alone or methyl esterification had no effect (Fig. 1). In contrast, the results of parallel experiments showed that the methyl ester of 5-HETE had significantly less neutrophil chemotactic activity than 5-HETE at concentrations of 200–5,000 ng/ml, whereas acetyl-5-HETE expressed neutrophil chemotactic activity that ranged from 75–87%, 78–91%, and 82–94% that of 5-HETE at concentrations of 200, 1,000 and 5,000 ng/ml, respectively. The presence of minimally chemotactic concentrations of acetyl-LTB4 suppressed the chemotactic responses to LTB4 in proportion to the molar ratio of the factors (Fig. 2), but had no effect on the responses to chemotactic fragments of C5 or to f-Met-Leu-Phe. At an equimolar concentration, acetyl-LTB4 inhibited by ∼50% the chemotactic responses to 1 ng/ml and 4 ng/ml of LTB4. Incubation of portions of neutrophils with 4 ng/ml and 10 ng/ml of acetyl-LTB4, followed by washing, failed to suppress the subsequent chemotactic response to 1 ng/ml of LTB4 relative to neutrophils preincubated in buffer alone. LTB4-methyl ester had only a minimal inhibitory effect that reached 20% at a 10-fold ratio of LTB4-methyl ester:LTB4.

Discussion

LTB4 elicits a maximal neutrophil chemotactic response and a response that is one-half of the maximal level at concentrations of ~10⁻⁷ M and ~6 × 10⁻⁸ M, respectively (Fig. 1), which are similar in magnitude to those evoked by 4 × 10⁻⁶ M and 4 × 10⁻⁷ M 5-HETE (13), and by 1.7 × 10⁻⁸ M and 1.3 × 10⁻⁹ M C5a (14). The neutrophil chemotactic activity of acetyl-LTB4 is significantly less than that of LTB4 at concen-
The availability of a series of other highly purified di-HETE, that were generated by neutrophils, permitted an analysis of the functional role of the double bonds in the conjugated triene of LTB₄. The triene of LTB₄, as for that of LTC₄ and LTD₄, consists of one cis- and two trans- double bonds, but the positions of the bonds have not been established unequivocally for LTB₄ (3, 5, 6). The 5(S),12(R)-all-trans-di-HETE isomer, which differs from LTB₄ only in having a trans-double bond in place of a cis-double bond in the triene portion of the molecule (6), is significantly less potent than LTB₄, but evokes a maximal chemotactic response of similar magnitude (Fig. 1). The cis-double bond and the free hydroxyl-group(s) thus appear to be critical determinants of the potency of LTB₄, but are not required to attain maximal activity unlike the free carboxyl-group of 5-HETE. Although the other 5,12-di-HETE and both 5,6-di-HETE isomers also were less potent than LTB₄, differences in the conformation or position of the second hydroxyl-group as well as in the structure of the triene may have contributed to the altered function.

The capacity of minimally chemotactic concentrations of acetyl-LTB₄ to inhibit selectively the human neutrophil chemotactic response to LTB₄ is reminiscent of the competitive inhibition of the chemotactic responses to 5-HETE by 5-HETE methyl ester (9) and to f-Met-Leu-Phe by several chemotactically inactive peptide analogues (15, 16). That the washing of neutrophils that had been preincubated with acetyl-LTB₄ fully reversed the suppression of the chemotactic response to LTB₄ suggests a competitive mechanism of inhibition. The results of competitive binding studies indicate that some inactive peptide analogues inhibit the chemotactic and other
activities of f-Met-Leu-Phe by displacing the stimulus from the receptor, but comparable binding data are not yet available for LTB₄ or the mono-HETE.

Summary

A specific 5(S),12(R)-dihydroxy-eicosa-6,8,10(trans/trans/cis), 14(cis)-tetraenoic acid, designated leukotriene B, is generated by the lipoxygenation and subsequent enzymatic hydration of arachidonic acid in a variety of leukocytes. Leukotriene B elicits a maximal human neutrophil chemotactic response in vitro which is similar in magnitude to those evoked by the chemotactic fragment of the fifth component of complement, C₅a, synthetic formyl-methionyl peptides, and 5-hydroxy-eicosatetraenoic acid (5-HETE). The neutrophil chemotactic potency of purified leukotriene B, assessed by the 50% effective concentration of $6 \times 10^{-9}$ M, is equivalent to that of C₅a, but is up to 100-fold greater than that of 5-HETE and of other natural di-HETE isomers. 5(S),12(R)-di-hydroxy-eicosa-6,8,10(all-trans),14(cis)-tetraenoic acid, which differs from leukotriene B only in having a trans-double bond in place of a cis-double bond in the triene portion of the molecule, and acetyl-leukotriene B are significantly less potent neutrophil chemotactic factors than leukotriene B, which indicates that both the conjugated double bonds and the free hydroxyl-group(s) are functionally critical determinants. The capacity of acetyl-leukotriene B to inhibit competitively and selectively the human neutrophil chemotactic response to equimolar concentrations of leukotriene B suggests the existence of a specific subset of receptors for this potent lipid mediator.

Received for publication 2 October 1980 and in revised form 1 November 1980.

References

activity of arachidonic acid lipoygenase products on leucocytes of different species. Prostaglandins. 20:411.