

STIMULATION OF HUMAN LEUKOCYTE DEGRANULATION BY LEUKOTRIENE B₄ AND ITS ω -OXIDIZED METABOLITES

Steven J. FEINMARK, Jan Åke LINDGREN, Hans-Erik CLAESSION, Curt MALMSTEN and Bengt SAMUELSSON

Department of Physiological Chemistry, Karolinska Institutet, 104 01 Stockholm, Sweden

Received 2 November 1981

1. Introduction

Leukotriene (LT)₄ has been indicated to play an important role in inflammatory reactions such as leukocyte chemotaxis [1,2] and adhesion to post-capillary venules [3]. In [4–6] LT₄ was suggested to be a weak stimulator of polymorphonuclear leukocyte (PMNL) degranulation. However, it is now clear that previous purification procedures for biosynthetically prepared LT₄ were inadequate. The newly discovered LT₄ isomer, 5(*S*),12(*S*)-DHETE, co-chromatographs with LT₄ in the reverse phase high-pressure liquid chromatography (HPLC) systems commonly used [7]. As a consequence, earlier studies probably tested preparations which contained a mixture of both isomers with the non-leukotriene product predominating. Therefore, we have investigated the role of LT₄, 5(*S*),12(*S*)-DHETE and their ω -oxidation products (fig.1) [8] in leukocyte degranulation *in vitro*. In addition, the activity of the non-enzymatically formed LT₄ isomers was investigated.

This study shows that LT₄-stimulated degranulation at 10⁻⁸ M; the ω -oxidation products of LT₄ were less potent while 5(*S*),12(*S*)-DHETE had no activity even at 2 × 10⁻⁶ M. Furthermore, the latter compound inhibited enzyme release induced by LT₄.

Abbreviations: 5(*S*),12(*S*)-DHETE, 5(*S*),12(*S*),dihydroxy-6-*trans*,8-*cis*,10-*trans*,14-*cis*-eicosatetraenoic acid; fMLP, *N*-formyl-methionyl-leucyl-phenylalanine; LT₄, 5(*S*),12(*R*)-dihydroxy-6-*cis*,8,10-*trans*,14-*cis*-eicosatetraenoic acid; 20-OH-LT₄, 5(*S*),12(*R*),20-trihydroxy-6-*cis*,8,10-*trans*,14-*cis*-eicosatetraenoic acid; 20-COOH-LT₄, 5(*S*),12(*R*)-dihydroxy-6-*cis*,8,10-*trans*,14-*cis*-eicosatetraen-1,20-dioic acid; 5(*S*),12(*S*),20-THETE, 5(*S*),12(*S*),20-trihydroxy-6-*trans*,8-*cis*,10-*trans*,14-*cis*-eicosatetraenoic acid

2. Materials and methods

Cytochalasin B, phenolphthalein glucuronic acid, and dried *Micrococcus lysodeikticus* were purchased from Sigma (St Louis MO). Dextran T-500 was obtained from Pharmacia Fine Chemicals (Uppsala) and fMLP was a product of UCB SA (Brussels).

2.1. Cell preparation and incubations

Purified polymorphonuclear leukocytes were prepared from leukocyte concentrates of human peripheral blood (Karolinska Hospital, Stockholm) by dextran sedimentation followed by hypotonic lysis of residual erythrocytes [9]. The cells were diluted to 5 × 10⁶ cells/ml in Dulbecco's phosphate-buffered saline (pH 7.4). Aliquots (1–2 ml) of the cell suspension were preincubated for 5 min with cytochalasin B (5 μ g/ml in DMSO 1 μ g/ml) in plastic vessels at 37°C. Test substances were added as free acids in either ethanol, methanol or DMSO. At the levels used, these solvents had no effect on spontaneous release. Incubations were terminated after 5 min by transfer to an ice bath followed by rapid centrifugation. The cell-free supernatants were subsequently assayed for the presence of lysozyme, β -glucuronidase and lactic acid dehydrogenase (LDH).

2.2. Enzyme assays

β -Glucuronidase was measured by a modification of the procedure in [10]. Supernatant (0.2 ml) was added to 2 ml acetate buffer (0.1 M, pH 4.6) followed by 0.1 ml phenolphthalein glucuronic acid (0.01 M, pH 7.0) as substrate. The reaction was terminated after 18 h at 37°C by the addition of 5 ml glycine buffer (0.2 M in 0.2 M NaCl, pH 10.4). The absorb-

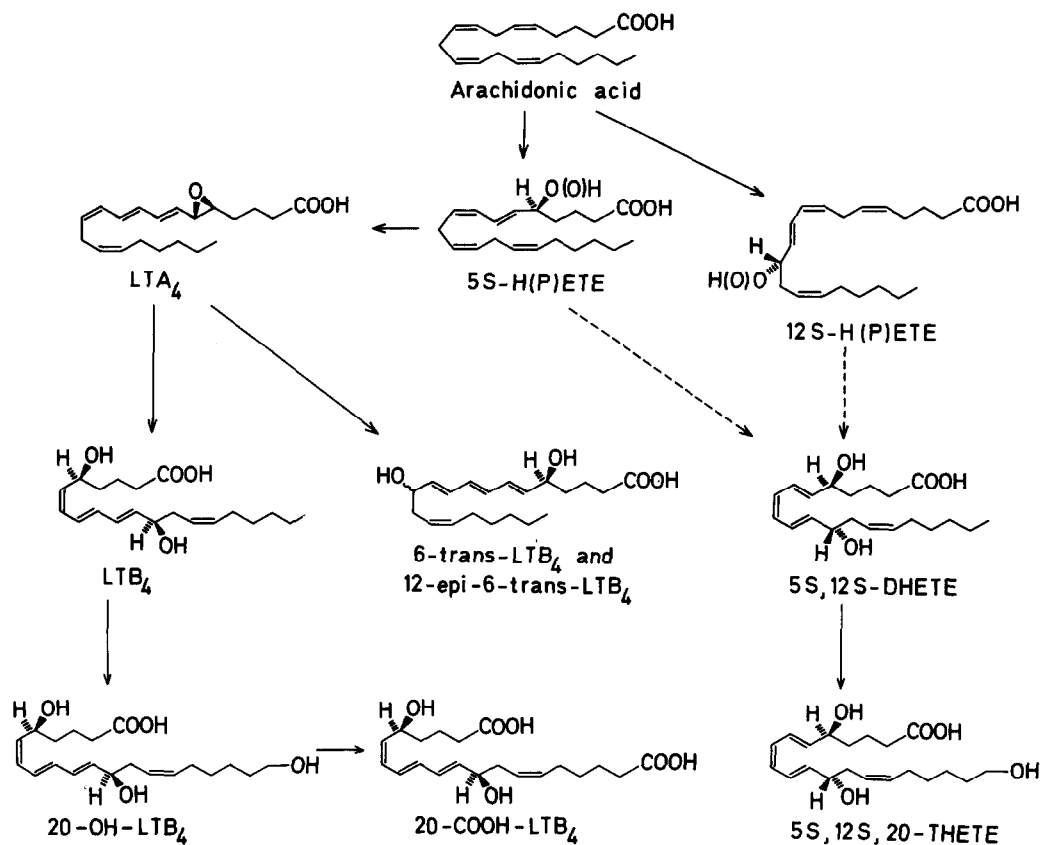


Fig.1. Products of arachidonic acid metabolism by human polymorphonuclear leukocytes: (--->) proposed conversions.

ance of each sample was determined at 540 nm vs a glycine buffer blank.

Lysozyme was determined by a turbidimetric assay [11] based on the ability of this enzyme to lyse suspensions of *Micrococcus lysodeikticus*. Supernatant (0.2 ml) was added to 0.8 ml *M. lysodeikticus* suspension (0.03 mg/ml in 0.1 M phosphate buffer, pH 7.0) and the fall in absorbance was monitored at 450 nm in a Cary 219 recording spectrophotometer.

LDH was monitored according to [12] and never significantly exceeded control levels.

The obtained enzyme release was corrected for spontaneous release and then calculated as a percentage of the total cellular enzyme content. This was determined by assaying the 10 000 × g supernatant of sonicated cell suspensions.

2.3. Leukotriene preparation

LTB₄, 5(S),12(S)-DHETE [7], 6-trans-LTB₄, 12-epi-6-trans-LTB₄ [13] and 20-OH-LTB₄ [8] were

prepared as reported. 20-COOH-LTB₄ was prepared by a further purification of the major polar fraction in [8]. This fraction was subjected to reverse-phase HPLC using methanol/water/acetic acid (50:50:0.01, by vol.) followed by rechromatography eluting with methanol/water/acetic acid (45:55:0.01, by vol.). The purity of this compound was verified on straight-phase HPLC [8] after conversion to the dimethyl ester.

3. Results

A series of arachidonic acid metabolites was tested for the ability to induce degranulation in cytochalasin B-treated human PMNL in vitro. The compounds studied were LTB₄, 20-OH-LTB₄, 20-COOH-LTB₄, 6-trans-LTB₄, 12-epi-6-trans-LTB₄, 5(S),12(S)-DHETE and 5(S),12(S),20-THETE. LTB₄ and its metabolites, 20-OH-LTB₄ and 20-COOH-LTB₄, induced the release of both lysozyme and β-glucuronidase (fig.2). 6-trans-

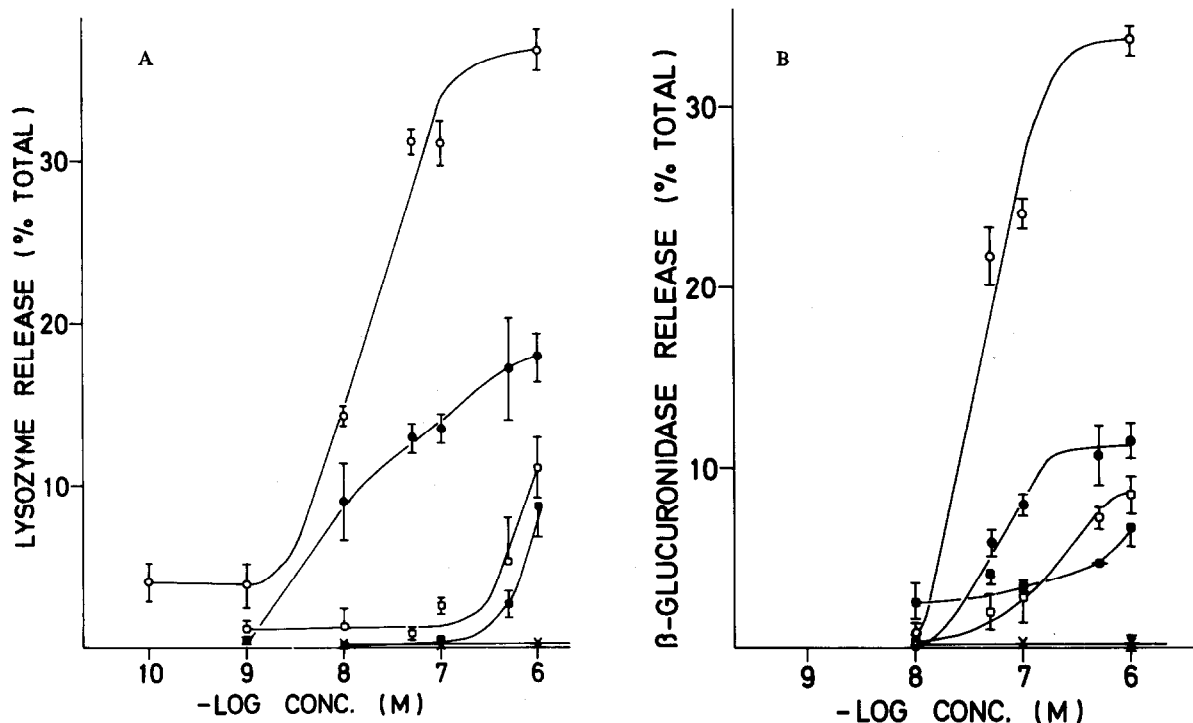


Fig.2. The release of granular enzymes from human polymorphonuclear leukocytes stimulated with varying concentrations of LTB₄ (●), 20-OH-LTB₄ (◻), 20-COOH-LTB₄ (◼), 5(S),12(S)-DHETE (×) and fMLP (○). Error bars denote standard errors of the mean of triplicate observations: (A) lysozyme; (B) β-glucuronidase. The curves representing fMLP-induced release are presented for comparison to other studies in which this agent is frequently used. Although only a few points are plotted, the shape of the curve is supported by data in [14,15].

LTB₄, 12-epi-6-*trans*-LTB₄, 5(S),12(S)-DHETE and 5(S),12(S),20-THETE were all unable to induce enzyme release. LTB₄ was found to yield a half-maximal release of lysozyme at 3.5×10^{-8} M (mean of 4 expt). 20-OH-LTB₄ and 20-COOH-LTB₄ were able to stimulate enzyme release equal to half the LTB₄ maximum only at levels 10–100-times higher (on a molar basis). At 5×10^{-7} M, the LTB₄-induced lysozyme release was 17.2%. When added at the same molar concentration, 20-OH-LTB₄ and 20-COOH-LTB₄ caused 5.3% and 2.6% release, respectively.

The activity of LTB₄ was compared to that of the chemotactic peptide, fMLP. Maximal enzyme release induced by LTB₄ was 35–50% of the enzyme released by fMLP.

5(S),12(S)-DHETE has been found to make up a large (60–75%) fraction of the 'LTB₄-peak' isolated from reverse-phase HPLC [7]. Therefore, the possibility that the two isomers interacted to alter the activity of LTB₄ alone was tested. 5(S),12(S)-DHETE, at

10^{-6} M, inhibited half-maximal LTB₄ stimulation of lysozyme release by 42% ($p < 0.001$). Furthermore, this concentration of 5(S),12(S)-DHETE had no effect on the release of lysozyme induced by fMLP.

4. Discussion

Several groups have investigated the enzyme-releasing effects of LTB₄ on peripheral leukocytes. LTB₄ was found to induce degranulation of cytochalasin B-treated human peripheral neutrophils but this occurred at a half-maximal dose of $\geq 10^{-6}$ M [4,6]. Earlier biosynthetic LTB₄ preparations obtained from human leukocyte suspensions contained a non-leukotriene isomer, 5(S),12(S)-DHETE [7]. Although the ratio of these two conjugated trienes is variable, 5(S),12(S)-DHETE can make up considerably >50% of the ultraviolet light-absorbing material eluting in the 'LTB₄-peak' from reverse-phase HPLC. These

isomers can now be separated using straight-phase HPLC [7]. It was therefore of interest to re-evaluate the biological activity of LTB₄, 5(S),12(S)-DHETE and the mixture of the 2 compounds.

LTB₄ was found to be a potent inducer of enzyme release in human polymorphonuclear leukocytes. When added at as low as 10⁻⁸ M, LTB₄ caused the release of significant amounts of lysozyme. The ω-oxidized metabolites, 20-OH-LTB₄ and 20-COOH-LTB₄ were much less potent, indicating that ω-oxidation may be a first step of biological inactivation of LTB₄.

The maximal amount of enzyme released by LTB₄ was always lower than the amount released from fMLP-stimulated cells. Thus, LTB₄ is roughly 50% as efficacious as the chemotactic peptide (fig.2). This difference does not seem to be due to a selective discharge of one granule-type since markers for both azurophil (lysozyme) and specific granules (β-glucuronidase) yielded similar results. Instead, this observation suggests that different receptor populations may be involved.

5(S),12(S)-DHETE did not stimulate enzyme release. 6-*trans*-LTB₄, 12-*epi*-6-*trans*-LTB₄ and 5(S),12(S),20-THETE were also inactive in this respect. This shows that LTB₄-induced degranulation is a highly stereospecific effect. Similar observations have been made for LTB₄-induced chemotaxis [2]. However, at high relative concentrations, 5(S),12(S)-DHETE can interfere with LTB₄ activity. This competition, presumably at a cell-surface receptor, results in an observed inhibition of LTB₄-induced enzyme release. Previous studies, therefore, probably underestimated the potency of LTB₄ in two important ways:

- (i) The concentration of ultraviolet light-absorbing material included large amounts of 5(S),12(S)-DHETE, which was biologically inactive;
- (ii) This compound acted as a competitive antagonist of the LTB₄-induced enzyme releasing activity.

This report indicates that LTB₄ is sufficiently potent to play an important role in leukocyte degranulation *in vivo*. Further studies to clarify this role are in progress.

Acknowledgements

We thank Ms Inger Tollman-Hansson and Ms Margareta Hovgard for their excellent technical assistance. This study was supported by grants from Riksföreningen mot Reumatism, Konung Gustaf V:s 80-årsfond, M. Bergvalls Stiftelse, the World Health Organization, the Swedish Medical Research Council (proj. 03X-217) and the North Atlantic Treaty Organization.

References

- [1] Ford-Hutchinson, A. W., Bray, M. A., Doig, M. V., Shipley, M. E. and Smith, M. J. H. (1980) *Nature* 286, 264–265.
- [2] Malmsten, C. L., Palmblad, J., Udén, A. M., Rådmark, O., Engstedt, L. and Samuelsson, B. (1980) *Acta Physiol. Scand.* 110, 449–451.
- [3] Dahlén, S.-E., Björk, J., Hedqvist, P., Arfors, K.-E., Hammarström, S., Lindgren, J. Å. and Samuelsson, B. (1981) *Proc. Natl. Acad. Sci. USA* 78, 3887–3891.
- [4] Hafström, I., Palmblad, J., Malmsten, C. L., Rådmark, O. and Samuelsson, B. (1981) *FEBS Lett.* 130, 146–148.
- [5] Bokoch, G. M. and Reed, P. W. (1981) *J. Biol. Chem.* 256, 5317–5320.
- [6] Goetzl, E. J. and Pickett, W. C. (1980) *J. Immunol.* 125, 1789–1791.
- [7] Lindgren, J. Å., Hansson, G. and Samuelsson, B. (1981) *FEBS Lett.* 128, 329–335.
- [8] Hansson, G., Lindgren, J. Å., Dahlén, S.-E., Hedqvist, P. and Samuelsson, B. (1981) *FEBS Lett.* 130, 107–112.
- [9] Lundberg, U., Rådmark, O., Malmsten, C. and Samuelsson, B. (1981) *FEBS Lett.* 126, 127–132.
- [10] Talalay, P., Fishman, W. H. and Huggins, C. (1946) *J. Biol. Chem.* 166, 757–772.
- [11] Smolelis, A. N. and Hartsell, S. E. (1949) *J. Bacteriol.* 58, 731–736.
- [12] Wróblewski, F. and LaDue, J. S. (1955) *Proc. Soc. Exptl. Biol. Med.* 90, 210–213.
- [13] Borgeat, P. and Samuelsson, B. (1979) *J. Biol. Chem.* 254, 7865–7869.
- [14] Feinmark, S. J. (1981) unpublished.
- [15] Becker, E. L. (1976) in: *Molecular and Biological Aspects of the Acute Allergic Reaction* (Johansson, S. G. O. et al. eds) pp. 353–370, Plenum, New York.