Effects of novel leukotrienes on neutrophil migration

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1. INTRODUCTION

Arachidonic acid metabolites, formed via lipoxygenase pathways, constitute a novel class of mediators of inflammation and allergy [1]. Leukotriene B_4 (LTB_4) stimulates neutrophil migration, aggregation, adherence and, to a lesser extent, enzyme release [2–8].

LTB_4 can be further metabolized to a ω-hydroxylated compound (20-OH-LTB_4) and the corresponding dicarboxylic acid, (20-COOH-LTB_4) [9,10]. The initial enzymatic transformation of arachidonic acid in LTB_4-biosynthesis is lipoxygenation at C-5 [11]. However, formation of leukotrienes via 15-HPETE has also been reported, i.e., the formation of 8,15-LTB_4 and 14,15-LTB_4 [12,13]. By double dioxygenation, arachidonic acid is transformed to 5(*S*),12(*S*),-dihydroxy-6-*trans*,8-*cis*,10-*trans*,14-*cis*-eicosatetraenoic acid, (5(*S*),12(*S*)-DHETE) [14,15], which is also subjected to ω-oxidation [14]. Another compound formed by double dioxygenation is 5,15-DHETE [15,16].

This report concerns one aspect of the biological activities of these novel compounds, the migration stimulating ability for neutrophils in vitro. To assess whether possible interactions between the compounds and the substratum (or matrix) of the chemotaxis equipment could influence the results, we used two different techniques, the under agarose assay and the Boyden chambers.

Abbreviations: PMN, polymorphonuclear neutrophil granulocyte; LTB_4, leukotriene B_4 (5(*S*),12(*R*)-dihydroxy-6,8,10,14-eicosatetraenoic acid); 15-HPETE, 15-hydroperoxy-eicosatetraenoic acid; DHETE, dihydroxyeicosatetraenoic acid; fMLP, formyl-methionyl–leucyl–phenylalanine; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; HPLC, high performance liquid chromatography; GC–MS, Gas chromatography–massspectrometry; HBSS, Hank's balanced salt solution

2. MATERIALS AND METHODS

2.1. *Preparations of leukotrienes and related compounds*

All compounds tested in this study were obtained from incubations of human leukocytes. The leukocytes were prepared from white blood cell concentrates (buffy coat), kindly supplied by the Blood Central at the Karolinska Hospital. For preparation of cells see [12,17]. In short, the procedure involves dextran sedimentation, NH_4Cl-induced lysis of red cells and centrifugations. For incubation cells were suspended in Dulbecco's PBS, or in HBSS buffered with HEPES.

The cells were incubated with arachidonic acid, with or without ionophore A23187, or with the hydroperoxide 15-HPETE. To obtain LTB_4 [18] and the double lipoxygenation product 5(*S*),12(*S*)-DHETE [14,15] cells were incubated with arachidonic acid plus the ionophore A23187. These incubations also produce the ω-oxidation products (i.e., the 20-OH and the 20-COOH derivatives) of the respective dihydroxy acids [9,14]. Compounds thus obtained were purified by acidic extraction, column chromatography on silica gel and HPLC (straight phase and reverse phase). For separation of LTB_4 and 5(*S*),12(*S*)-DHETE straight phase HPLC of the methyl esters were required. The acid forms were regenerated by saponification

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and again purified on reverse phase HPLC.

A number of isomeric 8,15-dihydroxyderivatives and 14,15-dihydroxy derivatives or arachidonic acid are also formed in incubations of leukocytes with arachidonic acid and the ionophore A23187. Intact or disrupted leukocytes incubated with 15-HPETE give larger quantities of the 8,15- and the 14,15-dihydroxy acids [19].

The 5,15-DHETE studied here was obtained from incubations of human leukocytes with either 15-HPETE, and also with arachidonic acid plus ionophore A23187.

The identities of the above mentioned compounds were determined in the respective original presentations. When more material was prepared in new incubations, the identities were always assured by GC-MS or by proper chromatographic comparison with a known standard.

Quantitation of thus prepared leukotrienes was based on UV-spectroscopy. The absorption maxima around 270 nm were used, and an extinction coefficient of 40 000 has been assumed. In later determinations of LTB_4, however, the extinction coefficient 50 000 was used [20].

2.2. *Neutrophil function assays*

Twice washed, dextran-sedimented leukocytes, resuspended in Hank's balanced salt solution (HBSS) with 0.1% gelatin and 3.1% HEPES (Sigma, St Louis MO) to 1.2×10^9 neutrophil/l, were used for the agarose migration assay. Compounds studied (dissolved in 10% ethanol in HBSS) were added to the cytotaxin well, and migration by the leading front cells was measured as in [5].

Migration was also assessed by a modification of the Boyden chamber technique [21], where the lipoxygenase products or fMLP (dissolved in 0.1% ethanol in HBSS) were added to the attractant well, and neutrophils, prepared and suspended as above, but with the addition of 10% pooled human AB serum, to the upper compartment. After 3 h incubation, fixation and staining, the net no. neutrophils/10 randomly chosen high power (400×) magnification microscopic fields were enumerated by subtraction of the number of spontaneously moving cells. All analyses were carried out in du- or triplicates with leukocytes from 3–6 different donors.

3. RESULTS

The distances to the leading front cells in the agarose assay, after stimulation with the different lipoxygenase products or fMLP, are given in fig.1. It is clear that LTB_4 induces migration at lower concentrations than any of the other lipoxygenase products. However, fMLP is more potent than LTB_4, with regard to the maximal distance migrated by stimulated neutrophils and on a molar basis. Nonetheless, the lowest concentration at which a migratory response occurred was similar for LTB_4 and fMLP (5×10^{-9} M). Moreover, the dose–response range for fMLP is rather narrow compared with

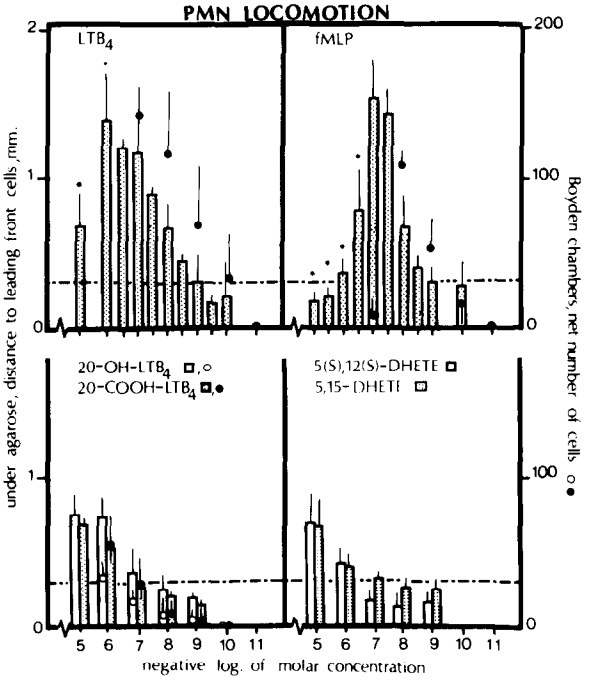

Fig.1. The distance to the leading front cells in the agarose assay (bars) and the net number of cells in the Boyden chambers (●,○) after stimulation with the lipoxygenase products and fMLP. Mean and SD values: (·····) mean + 2 SD value for spontaneously moving neutrophils under agarose, being 0.32 mm. Thus, all values exceeding this represent stimulated migration. The mean number (±SD) of spontaneously migrating cells in the Boyden chambers was 23 ± 17. (*) migratory pattern with the agarose assay, described as deactivation, where cells facing the cytotaxin well were densely packed, and cells facing the opposite well containing control media, appeared stimulated [2,27].

Table 1

Percentage of oriented cells after incubation for 45 min with lipoxygenase products as cytotaxins, at their optimal molar concentration, for stimulation of migration (Mean and SD values)

Compound	% oriented cells
LTB_4 (10^{-6} M)	51 ± 8
20-OH-LTB_4 (10^{-5} M)	46 ± 6
20-COOH-LTB_4 (10^{-5} M)	43 ± 7

The mean + 2 SD value for orientation of spontaneously moving cells is 30.9% [26]

that of LTB_4, partly depending on a marked deactivation migration pattern at concentrations in excess of 10^{-7} M. Of the other compounds tested here 20-OH-LTB_4 showed more activity than 20-COOH-LTB_4, which was followed by 5,15-DHETE and 5(*S*),12(*S*)-DHETE. The 8(*S*),15(*S*)-LTB_4 evoked a weak response at 10^{-5} M (0.33 ± 0.17 mm) but not at lower concentrations. 8(*R*),15(*S*)-LTB_4 exhibited no activity between 10^{-5}–10^{-10} M. LTB_4 and, to a lesser degree, 20-OH-LTB_4 and 20-COOH-LTB_4, evoked a chemotactic response, as judged from the degree of orientation of migrating neutrophils (table 1).

An essentially similar pattern of activity was found when the Boyden chamber technique was used (fig.1), although the peak activity occurred at ~10% of the concentration noted to be optimal in the agarose assay.

4. DISCUSSION

Neutrophils are the principal effector cells in host defense against bacteria and fungi as well as in inflammatory reactions. Soluble mediators generated at sites of infection and inflammation, such as bacterial and mitochondrial products (of which fMLP is a model substance) and activated serum complement factors (such as C5a) can activate neutrophil migration, adherence, aggregation, enzyme release, oxidative metabolism and microbicidal functions. These reactions are critical for host defence, but also contribute to tissue damage [22,23].

Lipoxygenase products, particularly LTB_4, are also potent stimulators of many but not all, neutrophil activities. Endogenous mediators could be the second messengers by which other inflammatory mediators, such as fMLP and C5a, exert a stimulatory effect on neutrophil functions. This hypothesis is supported by the finding that fMLP and serum-coated zymosan induce the synthesis of leukotrienes [10,24]. Investigations are in progress to elucidate whether these leukotrienes stimulate other activities of neutrophils, e.g., the oxidative metabolism.

It is interesting that 20-COOH-LTB_4, 20-OH-LTB_4 and LTB_4, in this order of potency, stimulated the contraction of guinea pig lung strips [9] whereas the opposite order of potency was true for stimulation of migration, as shown here. Moreover, leukotriene C_4 (LTC_4), being a slow reacting substance of anaphylaxis (SRS-A), is ~100-times more potent as constrictor of these lung strips as the LTB_4s, but lacks migration stimulating activity. It would hence seem conceivable that a balance exists between the migration and smooth muscle activities of the leukotrienes.

The two different chemotaxis assays yielded principally similar information. As noted by others, the optimal cytotaxin concentrations for a maximal migration response were ~10-times higher with the agarose assay compared with the Boyden chamber technique [25]. The reason for this discrepancy is probably due to the great difference in distance over which the gradient of the cytotaxin is generated, i.e., 2 mm in the agarose assay and 0.12 mm in a micropore filter. Since the under agarose assay also can be used to obtain information concerning, e.g., chemotaxis and chemokinesis [26], it is concluded that it is a valuable and versatile technique.

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REFERENCES

[1] Samuelsson, B. (1982) in: Advances in Prostaglandin, Thromboxane and Leukotriene Research (Samuelsson, B. and Paoletti, R. eds), vol. 9, pp. 1–17, Raven Press, New York.

[2] Malmsten, C., Palmblad, J., Udén, A.-M., Rådmark, O., Engstedt, L. and Samuelsson, B. (1980) Acta Physiol. Scand. 110, 449–451.

[3] Ford-Hutchinson, A.W., Bray, M.A., Doing, M.V., Shipley, M.E. and Smith, J.H. (1980) Nature 286, 264–265.

[4] Goetzl, E.J. and Pickett, W.C. (1980) J. Immunol. 125, 1789–1791.

[5] Palmblad, J., Malmsten, C., Udén, A.M., Rådmark, O., Engstedt, L. and Samuelsson, B. (1981) Blood 58, 658–661.

[6] Hafström, I., Palmblad, J., Malmsten, C., Rådmark, O. and Samuelsson, B. (1981) FEBS Lett. 130, 146–148.

[7] Bray, M.A., Cunninghamn, F.M., Ford-Hutchinson, A.W. and Smith, M.J.H. (1981) Brit. J. Pharmacol. 72, 483–486.

[8] 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.

[9] Hansson, G., Lindgren, J.Å., Dahlén, S.E., Hedqvist, P. and Samuelsson, B. (1981) FEBS Lett. 130, 107–112.

[10] Jubiz, W., Rådmark, O., Malmsten, C., Hansson, G., Lindgren, J.Å., Palmblad, J., Udén, A.-M. and Samuelsson, B. (1982) J. Biol. Chem. in press.

[11] Borgeat, P. and Samuelsson, B. (1979) Proc. Natl. Acad. Sci. USA 76, 3213–3217.

[12] Lundberg, U., Rådmark, O., Malmsten, C. and Samuelsson, B. (1981) FEBS Lett. 126, 127–132.

[13] Maas, R.L., Brash, A.R. and Oates, J.A. (1981) Proc. Natl. Acad. Sci. USA 78, 5523–5527.

[14] Lindgren, J.Å., Hansson, G. and Samuelsson, B. (1981) FEBS Lett. 128, 329–335.

[15] Borgeat, P., Fruteau de Laclos, B., Picard, S., Vallerand, P. and Sirois, P. (1982) in: Advances in Prostaglandin, Thromboxane and Leukotriene Research (Samuelsson, B. and Paoletti, R. eds) vol. 9, pp. 45–51, Raven Press, New York.

[16] Maas, R.L., Brash, A.R. and Oates, J.A. (1982) in: Advances in Prostaglandin, Thromboxane and Leukotriene Research (Samuelsson, B. and Paoletti, R. eds) vol. 9, pp. 29–44, Raven Press, New York.

[17] Borgeat, P. and Samuelsson, B. (1979) Proc. Natl. Acad. Sci. USA 76, 2148–2152.

[18] Borgeat, P. and Samuelsson, B. (1979) J. Biol. Chem. 254, 2643–2646.

[19] Rådmark, O., Lundberg, U., Jubiz, W., Malmsten, C. and Samuelsson, B. (1982) in: Advances in Prostaglandin, Thromboxane and Leukotriene Research (Samuelsson, B. and Paoletti, R. eds) vol. 9, pp. 61–70, Raven Press, New York.

[20] Corey, E.J., Marfat, A., Goto, G. and Brion, F. (1980) J. Am. Chem. Soc. 102, 7984–7985.

[21] Cates, K.L. (1981) in: Investigation of Phagocytes in Disease (Douglas, S.D. and Quie, P.G. eds) pp. 17–19, Churchill Livingstone, Londen.

[22] Weissmann, G., Korchak, H.M., Perez, H.D., Smolen, J.E., Goldstein, I.M. and Hoffstein S.T. (1979) J. Reticuloendothel. Soc. 26 suppl., 687–700.

[23] Goldstein, I.M. (1979) J. Lab. Clin. Med. 93, 13–16.

[24] Claesson, H.-E., Lundberg, U. and Malmsten, C. (1981) Biochem. Biophys. Res. Commun. 99, 1230–1237.

[25] Nelson, R.D., McCormack, R.T. and Fiegel, V.D. (1978) in: Leukocyte Chemotaxis (Gallin, J.I. and Quie, P.G. eds) pp. 25–42, Raven Press, New York.

[26] Palmblad, J., Udén, A.-M. and Venizelos, N. (1981) J. Immunol. Methods 44, 37–53.

[27] Chenoweth, D.E., Rowe, J.G. and Hugli, T.E. (1979) J. Immunol. Methods 25, 337–353.