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Biological properties of dihydro-leukotriene B_4, an alternative leukotriene B_4 metabolite

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Dihydro-leukotriene B_4 (a 5,12-dihydroxy-eicosatrienoic acid) has been shown to be the primary metabolite of leukotriene B_4 (LTB_4) in a variety of cells other than human polymorphonuclear leukocytes (PMNLs). In this report we show that dihydro-LTB_4 is significantly less active than LTB_4 in different biological assay systems, i.e. leukocyte chemotaxis, chemokinesis, aggregation, adhesion to endothelium and superoxide anion production. This suggests that primary reduction constitutes a second so far unknown deactivation pathway for LTB_4.

Leukotriene B_4; Dihydro-leukotriene B_4; Leukotriene metabolism; Chemotaxis; Oxidative burst; (Leukocyte)

1. INTRODUCTION

Leukotriene B_4 serves as an important endogenous mediator of inflammatory and immune reactions [1,2]. Its biological effects particularly concern responses of phagocytic leukocytes, such as chemotactic and chemokinetic movement, aggregation, adhesion to endothelial cells, increases in intracellular calcium levels, enzyme release by lysosomal degranulation, production of superoxide anion and hydrogen peroxide and actin polymerization [3–5]. It has been proposed that high- and low-affinity receptors are involved in most of these effects [6]. The LTB_4 receptor-mediated signal transduction is sensitive to pertussis toxin, thus indicating the existence of a guanine nucleotide regulatory protein controlling the LTB_4-induced cleavage of phosphatidylinositol 4,5-bisphosphate by phospholipase C [7–9] and subsequent translocation and activation of protein kinase C [10]. Little is known about the precise mechanisms of LTB_4 inactivation in vivo. We [11] and others [12] have previously reported that LTB_4 can be primarily transformed by reduction of a double-bond into a 5,12-dihydroxy-eicosatrienoic acid, termed dihydro-LTB_4, in different murine cells, including mesangial cells, fibroblasts and PMNLs, respectively. This represents an alternative metabolic pathway for LTB_4 in addition to the known ω-oxidation by human PMNLs, leading to 20-hydroxy/20-carboxy-LTB_4 (20-OH/20-COOH-LTB_4) [13]. In order to investigate whether the formation of dihydro-LTB_4 represents an effective deactivation step in LTB_4 metabolism, we compared the biological effects of LTB_4 and dihydro-LTB_4 in human leukocytes.

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Abbreviations: LTB_4, leukotriene B_4; dihydro-LTB_4, dihydroleukotriene B_4; PMNL, polymorphonuclear leukocyte; rp-HPLC, reverse-phase high-performance liquid chromatography

2. EXPERIMENTAL

2.1. *Preparation of dihydro-LTB_4*

Dihydro-LTB_4 was obtained through conversion of LTB_4 (kindly provided by Hoechst AG) by rat mesangial cells. For

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this purpose 10 μg LTB_4 together with trace amounts of [^{3}H]LTB_4 (Amersham) were added to 5×10^6 cells for 4 h under serum-free conditions. LTB_4 and its metabolites were purified by rp-HPLC and solid-phase extraction [11].

2.2. *Cells*

Human non-fractionated leukocytes were isolated from venous blood (mixed with 3 mmol/l Na_2-EDTA as anticoagulant) of healthy donors by dextran sedimentation [14]. The cells were finally suspended in phosphate buffered saline (1×10^6 cells/ml, superoxide anion production) or in complete Gey's solution (always containing 0.5% human serum albumin, grade 'purest', Behrinwerke) to final concentrations of 1×10^6 or 6×10^6 cells/ml (chemotaxis and adhesion assay, respectively) or in Ca/Mg-free medium to 10^7 cells/ml (aggregation assay). For determination of chemokinetic migration PMNLs were isolated by centrifugation of the blood in a discontinuous gradient of Percoll (Pharmacia) as in [15] and suspended in complete Gey's solution to a concentration of 5×10^5 cells/ml.

2.3. *Bioassays*

2.3.1. Chemotaxis

Chemotactic migration towards concentration gradients of LTB_4 and dihydro-LTB_4 was assayed in slightly modified Boyden chambers with cellulose nitrate filters of 3.0 μm pore size (Sartorius) and quantified by the 'leading front method' as described in [14] with $C5_a$-desarg (peptide derived from complement factor 5) as standard.

2.3.2. Chemokinesis

These experiments were performed in Sykes-Moore chambers at 37°C for 15 min with evenly distributed stimuli to induce undirected, chemokinetic migration [16].

2.3.3. Adhesion to guinea-pig aorta

Adhesion of PMNLs to endothelium of aortic strips was measured in a superfusion model [17]. Finally, the numbers of adhering PMNLs were counted under the microscope.

2.3.4. Aggregation

Assays of this cell function were performed in an aggregometer as detailed in [14]. In addition to the experimental procedure described there, 2 μg/ml cytochalasin B (Sigma) were applied to the samples of leukocyte suspension 3 min before Ca/Mg in order to enhance the aggregation response.

2.3.5. Superoxide anion production

This was estimated by measuring the change in ferricytochrome *c* reduction (Sigma, type III) at 550 nm [18] after 30 min.

3. RESULTS AND DISCUSSION

Several bioassays were carried out to evaluate the biological properties of the primary LTB_4 metabolite dihydro-LTB_4. Chemotaxis represents the most specific biological response of PMNLs to LTB_4, which displays a half-maximal effect at a concentration in the nanomolar range. Fig.1 shows

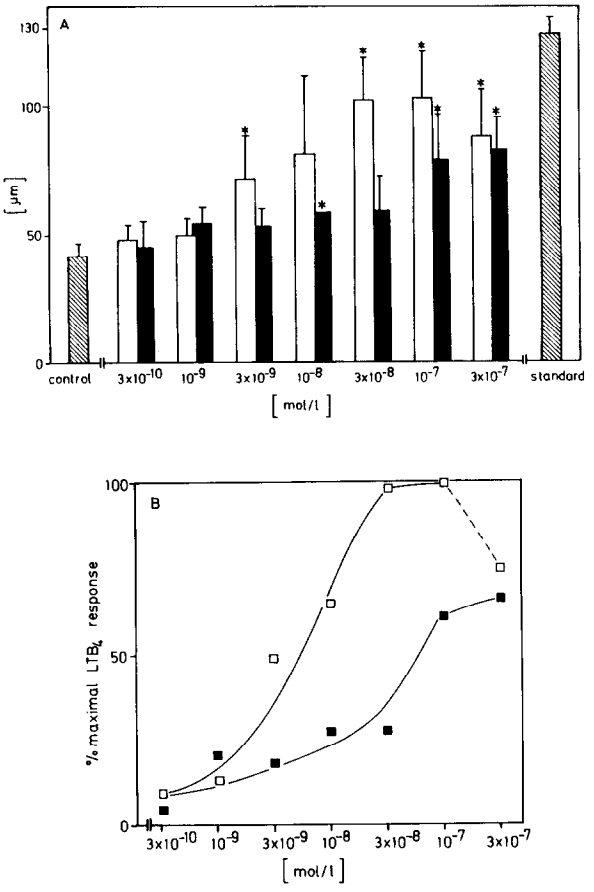

Fig.1. Effect of LTB_4 or dihydro-LTB_4 on human leukocyte chemotaxis. (A) LTB_4 (open bars) or dihydro-LTB_4 (closed bars) were used in the indicated concentrations. Results are the mean ± SD of 3 experiments with different cells, each comprising 5 determinations of the maximal distance of migration (μm). A Student's *t*-test was used to assess the significance of the chemotactic properties with * indicating $p < 0.05$. Control, spontaneous migration in the absence of a stimulus; standard, $C5_a$-desarg (1 μg/ml). (B) The mean values shown in A were transformed by subtracting the corresponding control values and related to the maximal LTB_4 response. LTB_4 (□), dihydro-LTB_4 (■).

that dihydro-LTB_4 exhibited a markedly lower chemotactic activity than LTB_4. The concentration of dihydro-LTB_4 required for half-maximal stimulation of PMNL chemotaxis was about 10–20-fold higher than that of LTB_4 (fig.1B). In comparison with LTB_4 a diminished biological activity of dihydro-LTB_4 was also observed in the stimulation of PMNL chemokinesis, adhesion to endothelial cells and aggregation (table 1). In all

Table 1

Effects of LTB_4 and dihydro-LTB_4 on human leukocyte functions

	Control	LTB_4	Dihydro-LTB_4	$C5_a$-desarg
Chemokinesis				
migration index	0	0.73	0.35	2.07
% LTB_4 response		(100)	(47.9)	(284)
Aggregation				
Δ transmission	0	8.7	3.2	nd
% LTB_4 response		(100)	(36.8)	
Adhesion				
cells/10 fields	30	58	42	nd
% LTB_4 response		(100)	(42.9)	

The effects of LTB_4, dihydro-LTB_4 and $C5_a$-desarg (10^{-7} mol/l each) on PMNL chemokinetic movement and on leukocyte aggregation and adhesion to endothelial cells, respectively, were determined as described in section 2. Results are means from duplicate determinations. nd, not determined

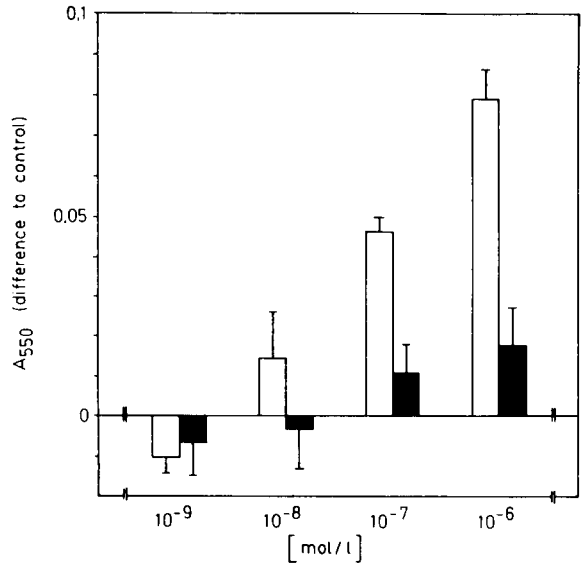

Fig.2. Effect of LTB_4 or dihydro-LTB_4 on human leukocyte oxidative burst. Superoxide anion production was measured as described in section 2. Values are the mean ± SD from triplicate determinations. LTB_4 (□), dihydro-LTB_4 (■).

these instances LTB_4 was less than half as active as LTB_4 at a concentration of 10^{-7} mol/l of both substances. The effectiveness of LTB_4 to stimulate PMNL chemokinesis was low compared to $C5_a$-desarg as standard, which was not the case with respect to the induction of PMNL chemotaxis (fig.1). It has been reported that LTB_4 can induce the oxidative metabolism of PMNLs [3]. This was confirmed in our experiments regarding the reduction of cytochrome *c* by superoxide anion after the addition of LTB_4 to leukocytes (fig.2). Again dihydro-LTB_4 was much less active. Even at a concentration of 10^{-6} mol/l no maximal response was achieved by both substances. This questions the in vivo relevance of these effects. Deoxyglucose uptake by leukocytes or macrophages could not be stimulated by LTB_4 or its metabolite (not shown).

Taken together, our results demonstrate that the conversion of LTB_4 into dihydro-LTB_4 causes a significant loss of biological activities of LTB_4. In the chemotaxis assay, which is the most specific biological test system for LTB_4, dihydro-LTB_4 exhibited less than 10% of the LTB_4 activity. Its effects on other PMNL functions were also lower than those of LTB_4 but could not be exactly quantified as for example superoxide anion production did not reach a maximum even at unphysiological concentrations (10^{-6} mol/l). Up to now two major routes for LTB_4 metabolism have been described. In human PMNLs 20-OH-LTB_4, which in some systems is as potent as LTB_4 itself [5], and 20-COOH-LTB_4 are formed [13], but the further degradation has to be carried out by other cells. In various murine cell types, including neutrophils [12], dihydro-LTB_4 is the primary LTB_4 metabolite, which can be converted to secondary hydrophilic products by these cells [11]. Our studies demonstrate that the primary reduction to dihydro-LTB_4 is an important alternative route of biological inactivation of LTB_4.

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