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SELECTIVE FEED-BACK INHIBITION OF THE 5-LIPOXYGENATION OF ARACHIDONIC ACID IN HUMAN T-LYMPHOCYTES

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SUMMARY

Purified human T-lymphocytes exhibit 5-lipoxygenase activity as demonstrated by the conversion of arachidonic acid to 5-hydroxy-eicosatetraenoic acid (5-HETE), 5(S),12(R)-di-hydroxy-eicosa-6,14 cis-8,10 trans-tetraenoic acid (leukotriene B_4), and 5,12-di-HETE isomers of leukotriene B_4 that lack a 6-cis double bond. The concentrations of leukotriene B_4, 5-HETE, 11-HETE and 15-HETE in suspensions of T-lymphocytes were increased significantly by concanavalin A and by the calcium ionophore A23187. Preincubation of T-lymphocytes with 15-HETE at μM concentrations, characteristic of suspensions of stimulated lymphocytes, inhibited selectively the increases in the levels of 5-HETE and leukotriene B_4, but not of 11-HETE and prostaglandin E_2.

INTRODUCTION

The 5-lipoxygenation of arachidonic acid is a quantitatively important pathway in many types of leukocytes, including basophils, polymorphonuclear (PMN)[1] leukocytes, monocytes and macrophages (1-6). 5(S)-hydroperoxy-eicosatetraenoic acid is the major initial product and is converted either to 5(S)-hydroxy-eicosatetraenoic acid (5-HETE) or to a series of complex 5(S)-hydroxy-metabolites, designated leukotrienes, that possess three conjugated double bonds and additional polar substituents. The leukotrienes are potent mediators of hypersensitivity and inflammatory reactions, as exemplified by the cysteine peptide-containing leukotrienes C_4 and D_4, which are the major

[1] Abbreviations used: PMN, polymorphonuclear; 5-HETE, 5(S)-hydroxy-eicosatetraenoic acid; SRS-A, slow-reacting substance of anaphylaxis; PG, prostaglandin; leukotriene B_4 or LTB_4, 5(S),12(R)-di-hydroxy-eicosa-6,14 cis-8,10 trans-tetraenoic acid.

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functional components of the slow-reacting substance of anaphylaxis (SRS-A), and by the di-hydroxy-derivative leukotriene B_4, which is a chemotactic factor for neutrophils and eosinophils (7-9). That endogenous lipoxygenase products of arachidonic acid also may be functional intraleukocyte constituents was suggested by the capacity of inhibitors of lipoxygenation to suppress a variety of leukocyte activities and by the finding that 5-HETE was capable, in some instances, of restoring the suppressed activities (10-13). The results of preliminary studies indicated that human peripheral blood lymphocytes possess a 5-lipoxygenase pathway which was stimulated by an unspecific mitogen (14). Diverse mono- and di-hydroxy-eicosatetraenoic acid products of the lipoxygenation of arachidonic acid in human T-lymphocytes are described in the present communication and the native 15-HETE is shown to inhibit selectively the activity of the 5-lipoxygenase, but not the cyclooxygenase, in the T-lymphocytes.

MATERIALS AND METHODS

Ficoll-Paque, high molecular weight dextran, and Sephadex G-10 (Pharmacia Fine Chemicals, Piscataway, N.J.), Hanks' balanced salt solution (Microbiological Associates, Bethesda, Md.), recrystallized concanavalin A and five-times recrystallized ovalbumin (Miles Laboratories, Inc., Elkart, Ind.), prostaglandin E_2 (PGE_2) (The Upjohn Co., Kalamazoo, Mich.), nylon wool (Leuko-Pak Leukocyte Filter, Fenwal Laboratories, Deerfield, Ill.), calcium ionophore A23187 (Calbiochem-Behring Corp., La Jolla, Calif.), arachidonic acid (Supelco, Inc., Bellefonte, Pa.), [^{3}H]PGE_2 and [^{3}H]arachidonic acid (New England Nuclear Corp., Boston, Mass.), and organic solvents that had been redistilled from glass (Burdick & Jackson Co., Muskegon, Mich.) were obtained from the designated sources.

Preparation of human T-lymphocytes. Venous blood from normal subjects was anticoagulated with sodium citrate and mixed with dextran to sediment the erythrocytes (8-10). The mononuclear leukocytes were purified by centrifugation of mixed leukocytes on Ficoll-Paque (8-10), washed and resuspended in Hanks' solution containing 0.4 g/100 ml of ovalbumin and 0.005 M Tris-HCl (pH 7.4), and filtered through 10 ml of Sephadex G-10 on a cushion of 4 ml of nylon wool (15). The lymphocytes obtained were free of platelets and other leukocytes and consisted of 77-86% T-lymphocytes, as judged by sheep erythrocyte rosetting, C3d receptor rosetting, and assessment of surface immunoglobulins (15).

Purification and characterization of the oxygenation products of arachidonic acid. T-lymphocytes were incubated for 20 min at 37°C at a concentration of 6-10 x 10^6/ml in Hanks' solution containing 0.4 g/100 ml of ovalbumin and 0.005 M Tris-HCl (pH 7.4) without or with 20 µg/ml of concanavalin A or 20 µM calcium ionophore A23187 and without or with 0.2 mg/ml of arachidonic

TABLE I

The Lipoxygenation of Arachidonic Acid in Human T-Lymphocytes

Conditions[a]							
Arachidonic Acid	Ca^{++} I A23187	Con A	15-HETE	11-HETE	5-HETE	LTB_4	"all-trans" 5,12-di-HETE[b]
			(ng/10^7 lymphocytes)				
0	0	0	364±202[c]	170±128	286±195	42±26	30±21
0	+	0	912±413	509±294	1038±437	193±114	139±99
+	0	0	7399±2716	3519±1710	3287±1461	383±172	245±190
+	+	0	38,421±7933	24,516±3958	20,347±7114	2518±839	2087±655
+	0	+	39,831±6494	21,927±4830	19,618±5272	2263±706	1834±591

[a]Suspensions of 0.6-1.0 x 10^7 lymphocytes were incubated for 20 min at 37°C without or with 0.2 mg/ml of arachidonic acid, 20 μM calcium ionophore A23187 (Ca^{++} I A23187) and 20 μg/ml of concanavalin A (con A).
[b]"All-trans" 5,12-di-HETE is a mixture of 5(S),12(R)-di-hydroxy-eicosa-6,8,10 trans-14 cis-tetraenoic acid and 5(S),12(S)-di-hydroxy-eicosa-6,8,10 trans-14 cis-tetraenoic acid, that were not resolved by the chromatographic procedure.
[c]Each value is the mean ± S.D. of the results of three experiments with lymphocytes from different donors.

acid. In some replicates, the lymphocytes were preincubated for 10 min at 37°C with lymphocyte-derived and purified 15-HETE or 11-HETE or with buffer alone prior to the addition of the other reagents and further incubation for 20 min at 37°C. Approximately 50,000 cpm each of [^{3}H]PGE_2 and of [^{3}H]5-HETE and [^{3}H]5(S),12(R)-di-hydroxy-eicosa-6,14 cis-8,10 trans-tetraenoic acid (leukotriene B_4 or LTB_4) that had been biosynthesized by human neutrophils and purified (9) were added to each tube. The products in the suspensions were extracted twice with two volumes of chloroform:methanol (2:1, v:v) and twice with two volumes of ethyl ether, and were resolved from residual arachidonic acid and more polar materials by silicic acid column chromatography (2,10). PGE_2, LTB_4, other di-hydroxy-eicosatetraenoic acids and the mono-HETEs were purified by reverse-phase high-performance liquid chromatography utilizing two different columns and solvent programs as described (9,16). The identity of each of the purified products was confirmed by ultraviolet light absorption spectroscopy and gas chromatography-mass spectrometry (2,9, 16). The quantities obtained were calculated from the optical density of each product at characteristic wavelengths of maximal absorption and were corrected for the overall losses estimated from the recovery of radioactivity corresponding to each internal standard.

RESULTS

Suspensions of purified human T-lymphocytes in buffer alone contained substantial concentrations of a variety of lipoxygenase products of arachidonic acid, of which 15-HETE and 5-HETE predominated quantitatively (Table I). LTB_4 and the 5,12-di-HETE isomers that differ from LTB_4 in lacking a cis-double bond in the triene portion of the molecule were present at concentrations approximately one-tenth those of the predominant mono-HETEs. The addition of the calcium ionophore A23187 increased the concentration of the

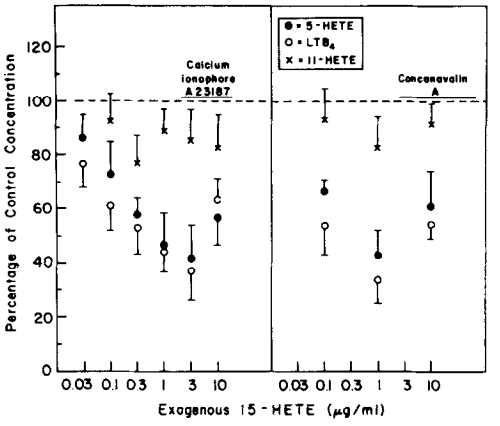

Figure 1 - Inhibition by 15-HETE of the 5-lipoxygenation of arachidonic acid in human T-lymphocytes. Each value is the mean ± S.D. of the results of three experiments, which is expressed as a percentage of the concentration of the respective HETE in control lymphocytes incubated with 0.2 mg/ml of arachidonic acid and the stimulus noted in the absence of exogenous 15-HETE. The difference between the value with 15-HETE and the control value is significant at $p < 0.01$ for: a) 5-HETE at concentrations of exogenous 15-HETE of 0.3 μg/ml or greater with calcium ionophore A23187 and of 0.1 μg/ml or greater with concanavalin A and b) LTB_4 at concentrations of exogenous 15-HETE of 0.1 μg/ml or greater with both stimuli (Student's t-test).

mono-HETEs by a mean of three-fold and of the di-HETEs by a mean of four- to five-fold (Table I). The introduction of exogenous arachidonic acid raised the concentrations of the di-HETEs by eight- to ten-fold and of the mono-HETEs by 15- to 20-fold, while the simultaneous addition of either calcium ionophore A23187 or concanavalin A further increased the concentration of all of the products by five- to eight-fold.

Lymphocytes were preincubated with different lipoxygenase products purified from other lymphocytes prior to the addition of arachidonic acid and either calcium ionophore A23187 or concanavalin A, in order to examine the possibility that the products might exert a feed-back inhibitory effect on the 5-lipoxygenase. 15-HETE inhibited in a dose-related manner the increases in concentration of 5-HETE and LTB_4, irrespective of the stimulus, with a maximal effect of approximately 60% inhibition at 1-3 μg/ml of 15-HETE and less inhibition at 10 μg/ml (Fig. 1). The increases in concentration of 11-HETE were not consistently influenced by 15-HETE (Fig. 1). Similarly, the increases in the concentration of PGE_2 were inhibited by a mean of 16%

or less by 0.3-3 μg/ml of 15-HETE (n=3). The preincubation of lymphocytes with 0.1-3 μg/ml of 11-HETE resulted in a mean maximal inhibition of the increases in concentration of 5-HETE and LTB_4 of only 20% and 21%, respectively (n=3).

DISCUSSION

The presence of a 5-lipoxygenase pathway in highly purified human T-lymphocytes has been established by the finding of both 5-HETE and a specific 5(S),12(R)-di-hydroxy-eicosa-6,14 cis-8,10 trans-tetraenoic acid, designated LTB_4, which is the product of enzymatic hydration of 5-hydroperoxy-eicosa-tetraenoic acid (Table I). The quantities of mono-HETEs and di-HETEs in suspensions of T-lymphocytes in buffer alone were similar to those detected in neutrophils (10) and lower than those in macrophages (6) in general, except that the concentrations of 5-HETE and 15-HETE were disproportionately high relative to that of 11-HETE when compared to the same ratios in the latter cell types. The capacity of concanavalin A, a soluble mitogenic stimulus, to increase the levels of the lipoxygenase products in T-lymphocytes to the same extent as the calcium ionophore A23187 differs from the effects of specific soluble stimuli in other leukocytes. The maximal elevation of the concentrations of lipoxygenase products of arachidonic acid in neutrophils and macrophages by specific chemotactic factors was only one-third or less of the increase achieved by the calcium ionophore A23187 in neutrophils and macrophages (6,10) or by phagocytosable particles in macrophages (6).

15-HETE, the quantitatively predominant mono-HETE in the T-lymphocytes, inhibited in a dose-related manner the increases in concentration of products of the 5-lipoxygenase in T-lymphocytes, without significantly influencing the increase in concentration of 11-HETE (Fig. 1) or of PGE_2. 15-HETE had been shown previously to inhibit the 12-lipoxygenase, but not the cyclo-oxygenase in platelets (17), at concentrations in the same μM range as those

required to inhibit the 5-lipoxygenase of T-lymphocytes (Fig. 1). The specificity of the effect of 15-HETE also was demonstrated by the failure of identical concentrations of 11-HETE to inhibit to a similar extent the 5-lipoxygenase of the T-lymphocytes. That the inhibitory capability of 15-HETE was manifested in the presence of excess exogenous arachidonic acid suggests that the site of action is the 5-lipoxygenase rather than the phospholipase which releases arachidonic acid.

Prostaglandins of the E series, that are produced by lymphocytes (18,19), bind to high affinity sites on the lymphocytes (20) and suppress both a wide range of effector functions (18,21) and the expression of specific receptors on the lymphocyte plasma membrane (22). As the rates of generation (14) and the concentrations of the lipoxygenase products in human lymphocytes (Table I) exceed those of prostaglandins, it will be of interest to investigate the effects on lymphocyte function of both exogenous HETEs and the inhibition of endogenous 5-lipoxygenase activity.

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