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EXPERIMENTS ON THE MODE OF ACTION OF PIRIPROST (U-60,257), AN INHIBITOR OF LEUKOTRIENE FORMATION IN CLONED MOUSE MAST CELLS AND IN RAT BASOPHIL LEUKEMIA CELLS

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Abstract—We studied the effect of piriprost, an inhibitor of sulfidopeptide leukotriene (LT) formation, on the generation of the known products of the 5-lipoxygenase pathway of arachidonate metabolism in calcium ionophore A23187-challenged rat basophil leukemia cells and cloned, growth factor-dependent, mouse mast cells. Piriprost inhibited the formation of 5-hydroxyeicosatetraenoic acid (5-HETE), and LTB_4, and the sulfidopeptide leukotrienes (LTC_4 in the mouse mast cells and both LTC_4 and a mixture of LTD_4 and LTE_4 in the rat basophil leukemia cells) in parallel (IC_{50} values ranged between 9 and 14 μM for the mouse mast cells and between 15 and 50 μM for the basophil leukemia cells). Our previous observation that piriprost is only a very weak inhibitor of the solubilized LTC synthase of rat basophil leukemia cells was extended to similar enzyme preparations derived from the mouse mast cells (IC_{50} 1.5 mM). The results are consistent with the conclusion that piriprost acts as an inhibitor of the 5-lipoxygenase reaction and that its activity in intact cells is not likely to involve the inhibition of the LTC synthase.

Leukotrienes (LT) are produced upon anaphylactic challenge of human and animal lung [1, 2], and exogenous administration of synthetic leukotrienes to laboratory animals [3, 4] or to humans [5–7] can mimic many of the symptoms of asthma. These two lines of evidence support the hypothesis that leukotrienes have a role in the pathogenesis of asthma. A key element in the proof of this hypothesis is still lacking, however: demonstration that selective inhibition of leukotriene formation or selective antagonism of the action of these mediators can have beneficial effects in patients suffering from this disease [8]. As a result, there is considerable theoretical interest in finding inhibitors that can be shown to inhibit selectively the formation of leukotrienes, so that the efficacy of such agents in the treatment of asthma can be tested.

We showed previously that piriprost [6,8-deepoxy-6,8-(*N*-phenyl)-imino-$\Delta^{6,9}$-prostaglandin I_1, U-60,257) is a selective inhibitor of sulfidopeptide leukotriene formation in ionophore A23187-challenged rat peritoneal mononuclear cells and in ragweed antigen challenged, IgE-sensitized, chopped human lung [9]. Subsequently, it was reported that similar concentrations of piriprost also inhibit the formation of LTB_4 in human polymorphonuclear leukocytes [10, 11] and the formation of sulfidopeptide leukotrienes in human eosinophils [12].

On the other hand, in our original description of the actions of this compound, we presented evidence that piriprost could inhibit the cytosolic glutathione *S*-transferases of rat liver and suggested that this effect might explain the mode of action of piriprost. Subsequent studies indicated that the LTC synthase, which is responsible for sulfidopeptide leukotriene biosynthesis in rat basophil leukemia cells, actually is a particulate enzyme [13] quite distinct from the cytosolic glutathione *S*-transferases, both in terms of substrate specificity and inhibition profile [14, 15]. Indeed, while piriprost proved to be a potent inhibitor of partially purified cytosolic glutathione *S*-transferases [16], it was inactive even at quite high concentrations when tested on the solubilized, particulate LTC synthase of rat basophil leukemia (RBL) cells [17].

In cultured, growth factor-dependent mouse mast cells, however, piriprost appeared to inhibit LTC_4 synthesis selectively under conditions where the synthesis of LTB_4 was uninhibited [18]. Because of our concern with the detailed mode of action of piriprost, and in particular the level of selectivity of its action, we wondered if the LTC synthase of cultured mouse mast cells might be sufficiently different from the corresponding enzyme in RBL cells to explain this apparently novel site of action for piriprost. The present paper summarizes our investigations of the mode of action of piriprost in inhibiting leukotriene formation in cloned, growth factor-dependent mouse mast cells.

MATERIALS AND METHODS

Chemicals. Concanavalin A (twice recrystallized) was purchased from Miles Laboratories, Elkhard IN; Triton X-100 from Eastman Kodak, Rochester, NY; arachidonic acid from NuCheck, Elysian, MN; A2318 from the Calbiochem Division of the American Hoechst Corp., San Diego, CA; 1,5-difluoro-2,4-dinitrobenzene, L-arginine, L-asparagine, and folic

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acid from the Sigma Chemical Co., St. Louis, MO; solvents for high pressure liquid chromatography from American Burdick & Jackson, Muskegon, MI; Eagle's Minimal Essential Medium, glutamine, sodium pyruvate, essential vitamin and non-essential amino acid solutions, penicillin, streptomycin and heat-inactivated newborn bovine serum from the Grand Island Biological Co., Grand Island, NY; Dulbecco Modified Eagle's medium from KC Biologicals, Lenexa, KS; gentamycin sulfate from Burns Biotech Laboratories, Omaha, NE; radioimmunoassay kits for the determination of LTB_4 from Amersham, Arlington Heights, IL; tritiated LTC_4 and LTD_4 from the NEN Division of E. I. du Pont de Nemours & Co., Boston, MA; and radioimmunoassay kits for the determination of 5-hydroxyeicosatetraenoic acid (5-HETE) from Seragen, Boston, MA. Piriprost, the lithium salt of LTA_4, LTC_4, LTD_4 and reference standards for the other potentially cross-reacting eicosanoids were prepared at the Upjohn Co. using published methods.

Culture conditions, solubilization of LTC synthase, and tests for leukotriene synthesis. RBL cells (ATCC CRL 1378) were cultured in Minimal Essential Medium (Eagle) supplemented with glutamine, antibiotics and 15% newborn bovine serum as previously described [14]. The cloned growth factor-dependent mast cells (1C9; [19] were derived from BALB/c mouse spleen cells according to methods reported in detail [20, 21]. The cells were maintained in suspension in Dulbecco's Modified Eagle's Medium which was supplemented with arginine, asparagine, folic acid, sodium pyruvate, essential vitamins, non-essential amino acids, glutamine, 2-mercaptoethanol, sodium bicarbonate, fetal calf serum and conditioned medium from concanavalin A-activated BALB/c spleen cells in a 5% CO_2, 95% air atmosphere as previously described [21, 22]. The 1C9 cells used for these experiments were strictly dependent on conditioned medium for survival and proliferation *in vitro* (doubling time approximately 24 hr) and were similar to other cloned or uncloned growth factor-dependent mouse mast cells reported by S. J. Galli in ultrastructure, histamine and serotonin content, ability to synthesize serotonin, and the number of surface receptors that bind to IgE with high affinity [19–22]. The suspending buffer which was used for the isolation of the LTC synthase and for its assay [137 mM NaCl, 2.6 mM KCl, 0.36 mM NaH_2PO_4, 10 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES), 1 mM EDTA, 0.3% Triton X-100, pH 7.0], as well as procedures used for the isolation of the particulate fraction from RBL cells and the solubilization of the LTC synthase, have been described [14]. 1C9 cells were washed in suspending buffer (without Triton X-100) and were suspended at a concentration of 10^8 cells/ml. However, because of limitations on the numbers of these cells which were available, the cells were broken by three cycles of freezing and thawing rather than by nitrogen cavitation. The broken cell preparations were processed as described for RBL cells.

Routine assays for ionophore A23187-induced leukotriene production by intact cells were carried out in challenge buffer [137 mM NaCl, 2.7 mM KCl, 0.36 mM NaH_2PO_4, 0.8 mM $CaCl_2$, 5 mM glucose, 10 mM HEPES, pH 7.0, 1 g/l bovine serum albumin (BSA)]. Because of the limited availability of the 1C9 cells, the incubation volumes and final cell concentrations for the 1C9 cells were reduced from 0.5 ml and 7.5×10^6 cells/ml, which was used for RBL cells, to 0.2 ml and 3×10^6 cells/ml. The cells, at 2 or 2.5 times the final concentration, were allowed to equilibrate at 30° for 20 min before being added to tubes containing aliquots of double-strength (or 2.5-times final strength for RBL cells) solutions of piriprost in buffer. L-Cysteine (10 μl of a 20- or 50-fold concentrated, neutralized solution) was then added to bring the concentration to 0.01 M, and this was followed 2.5 min later by addition of the ionophore in 10 μl (1C9 cells) or 100 μl (RBL cells). Incubation was continued for 20 min and was terminated by adding 4 vol. of ice-cold methanol. After standing at 4° for at least 30 min, the aggregated protein was removed by centrifugation, and the alcoholic supernatant solutions were evaporated to dryness on a SpeedVap dryer (Savant Instruments, Farmingdale, NY) which was thermostated at 45°. The dry samples were stored at −80° until assayed. Bovine serum albumin was used in place of the gelatin we ordinarily use in order to trap the 5-HETE and prevent its incorporation into cellular phospholipids [18]. The optimal ionophore concentration was determined empirically and was adjusted for the apparent decreased availability of this compound in the presence of bovine serum albumin (see specifics in Results).

Assays for LTC synthase activity were carried out in 0.1 ml of suspending buffer at a final glutathione concentration of 3 mM and a final LTA_4 concentration of 10 μM for an incubation period of 10 min at 37°. The reactions were terminated and processed as described above.

Assays for leukotrienes and for 5-HETE. The radioimmunoassays for LTB_4 and for 5-HETE were carried out with commercial kits exactly as specified by the manufacturers. The identity of the "LTB_4" which was being assayed with the LTB_4 radioimmunoassay was confirmed by high pressure liquid chromatography (HPLC) (see below). The radioimmunoassays for the sulfidopeptide leukotrienes employed antisera obtained from rabbits that had been immunized with LTC_4 or LTD_4 coupled to ovalbumin with a 2,4-dinitrobenzene linker as described by Young *et al.* [23]. The coupling ratios of the derivatized sulfidopeptide leukotrienes to the protein carrier were 3.41 and 3.58 mol/mol respectively, based on an assumed molar extinction coefficient of 27,000 at 344 nm for the dinitrobenzene linker. Primary immunization was with 1.5 mg of antigen in Complete Freund's Adjuvant (half intradermally in the back and half intramuscularly), followed 3 weeks later with 0.75 mg in Incomplete Freund's Adjuvant. Further booster immunizations (in the case of LTD_4) were given at monthly intervals and bleedings were taken 10–12 days after each boosting. The procedures for the radioimmunoassay were patterned after that previously described from our laboratories [24]. Free ligand was removed with 0.5 ml of a suspension of 0.5% charcoal and 0.05% dextran T-70 after an overnight incubation of radio-

labeled ligand, antibody, and unknown, in 0.2 ml of 0.01 M phosphate buffer, pH 7.4.

HPLC analysis of products formed in ionophore-challenged cells. The methanolic supernatant fraction from the cell incubations was evaporated to near-dryness (less than 10% of the original volume) and was diluted to 10 ml with HPLC grade water. These samples were passed through C18 Sep-Pak cartridges (Waters Associates, Milford, MA) that had been preconditioned by washings with 10 ml water, 10 ml methanol, 10 ml CH_2Cl_2, 20 ml methanol, 10 ml of 1 mM EDTA in water and 20 ml HPLC grade water. After rinsing of the cartridges with 20 ml water, the samples were eluted with 10 ml of 80% methanol and were concentrated to near dryness under N_2. The samples were reconstituted in 0.2 to 0.4 ml of 80% methanol, and aliquots were applied to an IBM reverse phase C18 column (Part No. SM3112943, IBM Danbury, CT) operating on a CCM (LDC/Milton Roy, Riviera Beach, FL) HPLC system. Elution was with an isocratic system consisting of H_2O–methanol–tetrahydrofuran–acetic acid (225:150:125:0.5) containing 44 mg/l EDTA, pH 5.3, at a flow rate of 1 ml/min. The system was calibrated with authentic eicosanoids and was employed for qualitative identification of the products generated in the cells.

RESULTS

Validation of the radioimmunoassays for LTC_4 and LTD_4. Typically, when 0.25 μl of antibody to LTC_4 was incubated with 0.56 pmol of tritiated LTC_4 (35.7 Ci/mmol), there was specific binding of 56% of the applied radioactivity. In the case of the antibody to LTD_4, 2.5 μl of antibody bound 35–45% of the 0.62 pmol of tritiated LTD_4 (specific activity, 40 Ci/mmol) which was added to the incubations. The specificity of the binding by these two antibodies is summarized in Table 1. Note that the only significant cross-reactivity for the antibody to LTC_4 which was found was with 11-*trans* LTC_4 (56%) and with LTD_4 (20%), whereas the antibody to LTD_4 had some cross-reactivity with LTC_4 (4% on a molar basis) and, more significantly, cross-reacted with LTE_4 (26%). The antibody to 5-HETE is reported by the manufacturer to react with the 5-HETE δ-lactone to the extent of 50%, with 5,15-DiHETE to the extent of 10%, with the 5-lipoxygenase-derived leukotrienes to the extent of 3–5% and with 15-HETE to the extent of 0.6%. The antibody to LTB_4 is reported to cross-react with other stereoisomers of 5,12-DiHETE (3.3%), diastereoisomers of 5,6-DiHETE (1.6%), 12-HETE (2%), 5-HETE (0.03%), LTC_4 and LTD_4 (0.03%), and 11-HETE (0.04%).

Table 1. Characterization of the specificity of the radioimmunoassays for LTC_4 and LTD_4

Competitor	% Cross-reactivity	
	Anti-LTC_4*	Anti-LTD_4*
LTC_4	100	4.0
11-*trans* LTC_4	56	0.7
LTD_4	20	100
11-*trans* LTD_4	1.1	15
LTE_4	0.09	26
11-*trans* LTE_4	0.27	0.26
LTB_4	0.11	0.15
5-HETE	0.04	0.09
15-HETE	0.04	<0.01
Glutathione	<0.000009	<0.00002

* Results are given as percent cross-reactivity on a molar basis and are based on the concentrations required to effect a 50% inhibition of binding of radiolabeled ligand as interpolated from concentration–response plots employing 2-fold incremental dilutions of the competing ligands.

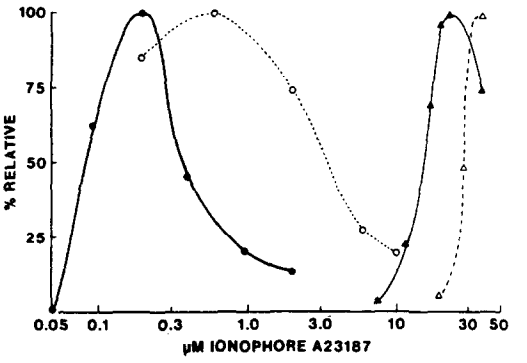

Fig. 1. Effect of ionophore concentration on the production of LTC_4 by 1C9 cells and of LTD_4 (+LTE_4) by RBL cells. The results are expressed relative to leukotriene production at the optimal ionophore concentration for each condition which is assigned the value of 100%. 100% values were 0.68 nmol/10^6 cells for 1C9 cells in medium without BSA (heavy solid line), 0.90 nmol/10^6 cells for 1C9 cells in BSA-containing medium (dotted line, open circles), 0.49 nmol/10^6 cells for RBL cells in challenge medium without BSA (expressed as LTD_4; light solid line, solid triangles) and 1.09 nmol/10^6 cells for RBL cells in BSA-containing medium (dashed line, open triangles). Results in each case are from two separate experiments in which all four conditions were compared using duplicate incubations for each ionophore concentration. For clarity, results for LTC_4 production by RBL cells are not shown though the optimal ionophore concentration was the same as that for LTD_4 production by these cells.

Determination of the optimal ionophore concentration. We have found that different strains of RBL-1 cells appear to differ in the concentration of the calcium ionophore which is required for the optimal production of leukotrienes. Previously we had employed a concentration of 3.3 μM A23187 for the optimal challenge of the strain of RBL cells originally received from Dr. T. Ishizaka, but we found that the cell line obtained from the American Type Culture Collection generated little if any leukotrienes when challenged with this concentration of the ionophore. Challenge of the 1C9 cells with this concentration of the ionophore also resulted in the generation of only small amounts of leukotriene C_4 and with killing of the cells as determined by trypan blue exclusion. As shown in Fig. 1, the optimal ionophore concentrations for the cells used in this study differed drastically. Furthermore, there was an approximately 2- to 3-fold shift in the optimal ionophore concentration when BSA was included in the challenge medium presumably because the

Table 2. Effect of piriprost on ionophore-induced production of 5-HETE, LTB_4, and LTC_4 in 1C9 cells*

	5-HETE	LTB_4	LTC_4
Uninhibited production† (pmol/10^6 cells)	3.5 ± 1.9	13.8 ± 6.4	414 ± 78
IC_{50} for piriprost (μM)			
Experiment I	8.7	5.9	6.8
Experiment II	12.2	11.0	9.9
IC_{50} after drug removal‡ (μM)	55	52	11

* See Materials and Methods for details.

† Results are the average of three independent experiments (quadruplicate incubations in each, ±SEM).

‡ Cells were incubated with piriprost at 30° for 5 min, centrifuged (600 rpm for 10 min), and the supernatant solutions were removed. The cell pellets were resuspended in fresh challenge buffer, replaced at 30°, and challenged after 2.5 min; results are from experiment I. In two other experiments, the IC_{50} was greater than the highest piriprost concentration which was tested (50 μM).

Table 3. Effect of piriprost on ionophore-induced production of 5-HETE, LTB_4, LTC_4 and LTD_4 in RBL cells*

	5-HETE	LTB_4	LTC_4	LTD_4
Uninhibited production† (pmole/10^6 cells)	0.64 ± 0.18	7.3 ± 2.4	189 ± 6.6	1089 ± 330
IC_{50} for piriprost‡ (μM)				
Experiment I	47.0	63.5	28.2	21.6
Experiment II	34.1	33.1	8.0	18.1
IC_{50} after drug removal‡§ (μM)				
Experiment I	111	207	129	65.9
Experiment II	74	127	79.7	85.9

* See Materials and Methods for details.

† Results are the average of three independent experiments (quadruplicate incubations in each, ± SEM).

‡ Results are from the same two experiments as described in Table 2, using quadruplicate incubations for each dosage point.

§ Cells were incubated with piriprost at 30° for 5 min and centrifuged (600 rpm for 10 min), and the supernatant solutions were removed. The cell pellets were resuspended in fresh challenge buffer, replaced at 30° and challenged after 2.5 min.

ionophore is bound to the BSA and is thus less available. The ionophore concentrations which were employed for subsequent studies were 0.18 and 0.61 μM for 1C9 cells in the absence and presence of BSA, and 27 and 34 μM for the RBL cells under the same two conditions.

Effect of piriprost on production of 5-lipoxygenase products by intact RBL and 1C9 cells. We tested the effect of piriprost on the formation 5-HETE, LTB_4 and LTC_4 in the 1C9 cells (Table 2) and the production of 5-HETE, LTB_4, LTC_4 and LTD_4 (or LTE_4) in the RBL cells (Table 3). In agreement with previous studies [25], we found in preliminary experiments (data not shown) that 1C9 cells generated predominantly LTC_4 with a small amount of LTB_4 and no significant amount of LTD_4, whereas the predominant product of RBL cells was LTD_4 (or LTE_4) with much less LTC_4, LTB_4 and 5-HETE. The HPLC analyses suggested that there was little if any 5,12-DiHETE produced other than LTB_4 by either cell. There was some variation in the 50% inhibitory concentration (IC_{50}) of piriprost for inhibiting the production of these metabolites from one experiment to the next. Furthermore, RBL cells were less susceptible to inhibition by piriprost than were 1C9 cells in the same experiment. However, the differences in the IC_{50} values for the inhibition of the formation of the different 5-lipoxygenase-dependent products in either cell type were not statistically significant (two-tailed *t*-test).

We reported previously [9] that inhibition of sulfidopeptide leukotriene formation by piriprost in ionophore-challenged rat peritoneal mononuclear cells did not require preincubation of the cells with the inhibitor and, furthermore, was rapidly reversed upon removal of the inhibitor from the cells. However, Razin *et al.* [18] later reported that, when bone marrow-derived cultured mouse mast cells were preincubated with piriprost for 5 min after sensitization with IgE, and were then challenged with

antigen after removal of the inhibitor, there was a selective inhibition of LTC_4 formation, while the production of LTB_4 returned to control levels. We therefore tested the persistence of the inhibition of leukotriene synthesis by piriprost in both RBL and 1C9 cells under conditions that were designed to reproduce those of Razin *et al.* It was necessary to use much higher piriprost concentrations during the preincubation period for any inhibition to persist after the cells had been centrifuged and resuspended in fresh buffer to remove the inhibitor. When the cells were washed once before resuspending them for challenge, as was done by Razin *et al.* [18], there was no residual inhibition of any of the end points being measured in cells that had been exposed to as much as ten times the IC_{50} concentration of piriprost. As might be expected, there was considerable variability in the persistence of the inhibition from one experiment to the next when the cells were resuspended and challenged with the ionophore without an intervening wash. This is probably a function of the small and variable amounts of piriprost that were carried over from the first incubation. In fact, even under these conditions, there was no persistent inhibition in several of the experiments (Table 2). We therefore do not believe that the IC_{50} values for the different end points that were obtained after the inhibitor had been removed have any real meaning.

Effect of piriprost on the solubilized LTC synthase. We previously reported [17] that piriprost does not inhibit the solubilized LTC synthase of RBL cells at concentrations up to 1 mM. In experiments not shown, this compound also failed to inhibit the particulate enzyme from RBL cells when it was tested without being solubilized and in the absence of Triton X-100. To determine if the LTC synthase of 1C9 cells might be more susceptible to inhibition by this compound, we prepared a solubilized enzyme preparation using the same procedures we described previously for the preparation of the solubilized enzyme from RBL cells [14], with the exception that cell breakage was achieved by freezing and thawing. The solubilized enzyme from 1C9 cells produced 0.88 pmol·mg protein^{-1}·min^{-1} of LTC_4 in our standard incubations, whereas we routinely observe LTC_4 production of 16.9 ± 1.8 pmol·mg protein^{-1}·min^{-1} with the enzyme from RBL cells. The IC_{50} for inhibiting the 1C9 enzyme by piriprost was 1.5 mM.

DISCUSSION

The overall intent of these studies was to test whether the inhibition of sulfidopeptide leukotriene production by piriprost in 1C9 cells can be explained by an inhibition of the 5-lipoxygenase, as it has in a number of other situations [9–12], or whether this compound inhibited the LTC synthase of these cells as was suggested by the experiments of Razin *et al.* [18]. Our studies did not address the possibility that the inhibition might take place on the mobilization of arachidonate from phospholipid pools. However, the observation that the production of cyclooxygenase-derived products can be even enhanced in the presence of this inhibitor [9] makes this explanation unlikely.

In confirmation of previous studies, we found that the sulfidopeptide leukotrienes were the major product of the 5-lipoxygenase pathway which were produced by both the 1C9 and the RBL cells. Of these, the 1C9 cells produced only LTC_4, whereas the major product of the RBL cells, in our studies, was LTD_4. There was considerable variation in the amount of LTC_4 that was produced by RBL cells and in the amounts of 5-HETE and LTB_4 that were found in the incubations of both cell types.

Piriprost inhibited the formation of all the 5-lipoxygenase products in both the 1C9 and the RBL cells. All these inhibitions were reversible in that none of them persisted when cells that had been preincubated with piriprost were washed once before they were challenged with the ionophore. Inhibition was reduced markedly upon the mere removal of the inhibitor. The IC_{50} for the inhibition of the formation of 5-HETE, LTB_4, and LTC_4 in 1C9 cells varied somewhat from one experiment to the next and also among the three end points in each of the experiments (Table 2). None of these differences was significant and, furthermore, there was no indication that the formation of LTC_4 was any more susceptible to inhibition than was the formation of LTB_4 or of 5-HETE. There was even more variability in the IC_{50} values for inhibiting the minor products of RBL cells (Table 3), but none of these differences was significant either. It is possible that this variability is related to the variation in the extent to which these substances are produced from one experiment to the next. Indeed, the experiments in which the IC_{50} was the furthest from a mean value for that cell line were those in which the production of a given product was the smallest.

Finally, piriprost only inhibited the crude LTC synthase of 1C9 cells at very high concentrations, a finding which is in agreement with our previous studies using the enzyme from RBL cells. In this regard, it should be stressed that even though the cloned mouse mast cells produced very large amounts of leukotrienes, the cell-free, solubilized LTC synthase from 1C9 cells appeared to be considerably less active than the enzyme we routinely obtain from RBL cells. The most likely explanation for this discrepancy may be the differences in the homogenization procedures used, and the fact that we did not ensure that the solubilization methods employed were optimal for these preparations. Optimal preparation of solubilized enzyme requires relatively large numbers of cells, and therefore large volumes of spleen cell-conditioned medium. However, our purpose was merely to establish an enzyme concentration-dependent synthesis, and then to test this activity for the effect of piriprost.

Thus, these results suggest that the inhibition of the formation of the products of the 5-lipoxygenase pathway in 1C9 cells occurs by the same mechanism as the one we had studied previously in mononuclear cells and in RBL cells. They do not support the existence of a second site of action and certainly not of a site for irreversible inhibition, as suggested by the results of Razin *et al.* [18]. The reasons for this difference in conclusions are not clear. However, there are several differences between our protocols and those of Razin *et al.* [18]. Most noteworthy

among these differences are that Razin *et al.* employed an IgE and antigen-dependent challenge of their cells whereas we used the calcium ionophore, and that Razin *et al.* used primary cultures of bone marrow-derived mast cells whereas we used a cloned mast cell line which was originally derived from mouse spleen cells. Thus, even though primary bone marrow-derived mast cells and cloned, growth factor-dependent mouse mast cells express a number of similarities (reviewed in Refs. 26 and 27), it is still possible that the bone marrow-derived mast cells studied by Razin *et al.* had an LTC synthase which was uniquely susceptible to inhibition by piriprost. Alternatively, or in addition, IgE and antigen, which is a more physiologic stimulus, might permit the appreciation of a subtle difference in the balance of the enzyme activities which are involved in arachidonate metabolism.

But other explanations for the result of Razin *et al.* [18] may be equally likely. For example, upon reversal of inhibition, the pool size of LTA_4 in the bone marrow-derived cells may have been just sufficient to sustain apparently uninhibited production of LTB_4, which was produced in relatively small quantities, whereas the amount of available LTA_4 was insufficient for the requirements of the LTC synthase. Information about the effect of piriprost on the formation of 5-HETE by the bone marrow-dependent mast cells of Razin *et al.* would be of interest, since such data may help to clarify the effect of the drug on these cells.

In conclusion, our results are consistent with the hypothesis that the inhibition of leukotriene formation by piriprost occurs at the 5-lipoxygenase step. Furthermore, cloned growth factor-dependent mouse mast cells are inhibited in a manner which is analogous to that seen in RBL cells. This further strengthens the impression that this is the mechanism of action of this compound in general. It would still be interesting to see if exceptions to this generalization can be found.

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