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EFFECTS OF METALLOPROTEINASE INHIBITORS ON LEUKOTRIENE A_4 HYDROLASE IN HUMAN AIRWAY EPITHELIAL CELLS

JOSEPH R. BAKER, TRIENEKE A. KYLSTRA and TIMOTHY D. BIGBY*

Department of Veterans Affairs Medical Center, San Diego, and the Department of Medicine of the University of California, San Diego, San Diego, CA, U.S.A.

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Abstract—Human neutrophil leukotriene A_4 (LTA_4) hydrolase is a zinc-containing metalloproteinase with aminopeptidase activity and can be inhibited by some metalloproteinase inhibitors. Human airway epithelial cells also contain an LTA_4 hydrolase enzyme that has some novel properties, suggesting that this enzyme may be functionally and structurally unique. Thus, we questioned whether the epithelial enzyme could also be inhibited by metalloproteinase inhibitors. Transformed human airway epithelial cells were studied either intact or disrupted. Of the metalloproteinase inhibitors examined, only captopril, bestatin, and fosinoprilat had appreciable inhibitory activity for LTA_4 hydrolase in disrupted epithelial cells. Concentration–inhibition curves to captopril, bestatin, and fosinoprilat revealed IC_{50} values of 430 μM, 7 μM, and 1 mM, respectively, for disrupted-cell LTA_4 hydrolase activity. In contrast to its effects on neutrophils, 1,10-*O*-phenanthroline had no significant effect on disrupted epithelial cell hydrolase activity and had only minimal effects when this activity was partially purified (179-fold). LTA_4 hydrolase concentration–inhibition curves examined in intact cells with captopril, bestatin, and 1,10-*O*-phenanthroline revealed IC_{50} values of 63, 70, and 920 μM, respectively. Aminopeptidase activity in disrupted epithelial cells was inhibited by amastatin, bestatin, and 1,10-*O*-phenanthroline (IC_{50} values of 500 nM, 1 μM, and 17 μM, respectively), but not by captopril at the highest concentration tested, 10 mM. These findings are in contrast to prior studies in neutrophils. When neutrophils were stimulated with A23187 after treatment with captopril, transcellular synthesis of LTB_4 was inhibited more effectively than direct synthesis of leukotriene B_4 (LTB_4) (43.8 ± 2.5 vs 18.5 ± 4.7%; N = 8, P < 0.02). We conclude that LTA_4 hydrolase activity of human airway epithelial cells is inhibited by some metalloproteinase inhibitors, but that the profile of inhibition is distinct from that for the neutrophil enzyme. These data provide additional information that LTA_4 hydrolase in the epithelial cell is a novel enzyme, distinct from that found in the neutrophil.

Key words: aminopeptidase; arachidonic acid; inflammation; lipoxygenase; transcellular metabolism

LTA_4† hydrolase is the enzyme responsible for metabolism of the unstable epoxide intermediate 5(*S*)-5,6,-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid, or LTA_4, to LTB_4 [1]. LTA_4 hydrolase has been purified to homogeneity from neutrophils [1], cloned [2], and expressed in bacteria [3]. This enzyme has a molecular mass of 69,140 Da, a pH optimum of 8, and undergoes suicide inactivation upon exposure to substrate by covalent linkage [3, 4]. LTA_4 hydrolase also has structural homology to zinc-containing metalloproteinases [5, 6] and has metalloproteinase activity of the aminopeptidase variety [7]. The aminopeptidase activity of LTA_4 hydrolase is enhanced by anions, such as chloride and bromide [8], or by albumins [9]. Further characterization of the neutrophil enzyme has demonstrated that it can be inhibited by metalloproteinase inhibitors, including captopril [10], bestatin [11], 1,10-*O*-phenanthroline [7, 12], and fosinoprilat [12]. Recent reports document the development of more potent inhibitors of purified neutrophil LTA_4 hydrolase of the α keto β amino ester or thioamine variety [13]. Captopril can also inhibit LTB_4 synthesis by intact neutrophils *in vitro* and *in vivo* [12]. Additional work *in vivo* has now demonstrated that bestatin can inhibit the formation of LTB_4 in isolated, perfused rat lungs [14].

Prior work from this laboratory has demonstrated that human airway epithelial cells contain LTA_4 hydrolase activity [15]. Studies with isolated cells suggested that the LTA_4 hydrolase activity present in airway epithelial cells might be different from the LTA_4 hydrolase enzyme characterized in neutrophils. This conclusion was based on a slower time course for product generation and lack of enzyme inactivation by substrate. Further characterization of this enzymatic activity in purified preparations from epithelial cells suggested that the airway epithelial cell enzyme is structurally related, but not identical, to the neutrophil enzyme [16]. Human airway epithelial cells contain a protein that is recognized by a polyclonal antibody raised to neutrophil LTA_4 hydrolase. However, this protein displays a cleavage pattern upon proteolytic digestion that is distinct from that obtained for the neutrophil enzyme. Further, epithelial LTA_4 hydrolase has a significantly different kinetic profile from that of the neutrophil enzyme, even after substantial (179-fold) purification, and is not saturable. In contrast to the neutrophil enzyme [7], the epithelial enzyme has little, if any, aminopeptidase activity.

Based on the described differences in LTA_4 hydrolase activity in neutrophils and airway epithelial cells, we questioned whether metalloproteinase inhibitors would be active in inhibiting epithelial LTA_4 hydrolase. Thus,

* Corresponding author: Timothy D. Bigby, M.D., 111J, Department of Veterans Affairs Medical Center, 3350 La Jolla Village Dr., San Diego, CA 92161. Tel. (619) 552-8585, Ext. 3541; FAX (619) 546-1754.

† Abbreviations: LTA_4, leukotriene A_4, 5(*S*)-5,6-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid; LTB_4, leukotriene B_4, 5(*S*),12(*R*)-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid; LTC_4, leukotriene C_4, 5(*S*)-hydroxy-6(*R*)-*S*-glutathionyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid; HBSS, Hanks' balanced salt solution; CMF-HBSS, calcium- and magnesium-free HBSS; and RP-HPLC, reversed-phase HPLC.

the purpose of these investigations was to determine if metalloproteinase inhibitors could modulate LTA_4 hydrolase activity in disrupted and intact human airway epithelial cells.

MATERIALS AND METHODS

Materials

HBSS and Minimum Essential Medium (MEM) were purchased from BioWhittaker Inc. (Walkersville, MD). LTA_4 ethyl ester was a gift of Merck Frosst Centre for Therapeutic Research (Pointe Claire-Dorval, Quebec, Canada). Redistilled-in-glass grade solvents were purchased from Burdick & Jackson, Baxter Scientific (Muskegon, MI). Percoll and Dextran 500 were purchased from Pharmacia LKB (Piscataway, NJ). The internal standard prostaglandin B_2 (PGB_2) was obtained from Upjohn (Kalamazoo, MI). Enalapril and enalaprilat were gifts from Merck, Sharp & Dohme (Rahway, NJ). Fosinopril and fosinoprilat were gifts from Bristol-Myers Squibb (Princeton, NJ). Quinapril and quinaprilat were gifts from Parke-Davis Pharmaceutical Research Division, Warner Lambert (Ann Arbor, MI). The remaining reagents were obtained from the Sigma Chemical Co., St. Louis, MO, and were of the finest grade available.

Cell and cell-free preparations

An immortalized human airway epithelial cell line, 9HTEo$^-$ (a gift of Dr. D. C. Gruenert, University of California, San Francisco), was utilized for these experiments. This cell line was used because prior studies have shown that this cell line has similar LTA_4 hydrolase activity when compared with primary cultures of human airway epithelial cells [15, 16]. Cells were grown to confluence on collagen, fibronectin, and BSA coated plastic in MEM with 10% fetal bovine serum (FBS). To prepare sonicates, confluent cells were washed twice with CMF-HBSS and then scraped from the tissue culture plastic using a rubber policeman into a buffer consisting of 20 mM KPO_4, 0.15 M NaCl, 5 mM EDTA, 50 mM Tris, pH 7.8. The cells were sonicated on ice using a Branson sonifier at power output of 2.5 for eight 5-sec pulses, 30 sec apart. Immediately after sonication, freshly prepared phenylmethylsulfonyl fluoride (PMSF) dissolved in absolute ethanol was added to a final concentration of 1 mM. Neutrophils were isolated from peripheral blood by previously described techniques [17] and were studied intact or sonicated as described for epithelial cells.

The 15,000 *g* supernatants of sonicated cells were used for most experiments. Previous studies have demonstrated that LTA_4 hydrolase is a cytosolic protein that resides exclusively in the supernatant when disrupted cells are subjected to centrifugation at 15,000 *g* [16]. Total protein was quantified by the Bradford microassay method [18], and the 15,000 *g* supernatant fraction was adjusted to a protein concentration of 3 mg/mL with 100 mM Tris, 1 mM EDTA, pH 7.8. The 15,000 *g* supernatant was flash-frozen in liquid nitrogen and stored at −70° until needed.

LTA_4 hydrolase assay

LTA_4 hydrolase assays were performed as previously described after dilution 1:1 in assay buffer (final concentration of 100 mM Tris, 0.15 M NaCl, 0.35% BSA, pH 7.8) [16]. Briefly, samples of 15,000 *g* supernatant were incubated with freshly hydrolyzed LTA_4 at a concentration of 15 μM for 15 min. The incubations were then quenched with methanol and acidified with HCl, and the samples were extracted as previously described [16].

Aminopeptidase assay

Aliquots of disrupted epithelial cells were mixed 1:1 with 2× assay buffer (final concentration 15 mM KH_2PO_4, 15 mM K_2HPO_4, 0.15 M NaCl, pH 7.0) and were prewarmed to 37°. L-Alanine-*p*-nitroanilide, dissolved in DMSO, was added to the samples to a final concentration of 1 mM. The DMSO concentration did not exceed 0.1%, and this concentration had no effect on enzymatic activity. Samples were incubated at 37° for 10 min in a temperature-controlled microplate reader (Thermomax, Molecular Devices, Menlo Park, CA). Enzymatic activity was assessed by monitoring the samples for release of the chromogenic product, *p*-nitroaniline, at 405 nm and quantifying product formation using its molar extinction coefficient (ε 9620) at pH 7.0.

Inhibition of LTA_4 hydrolase in disrupted airway epithelial cells

LTA_4 hydrolase assays were also performed on samples of 15,000 *g* supernatant in the presence of a broad range of metalloproteinase inhibitors. These inhibitors included amastatin, thiorphan, bestatin, 1,10-*O*-phenanthroline, EDTA, phosphoramidon, D-penicillamine, captopril, enalapril, enalaprilat, fosinopril, fosinoprilat, quinapril, and quinaprilat. Each inhibitor was added to aliquots of the 15,000 *g* supernatant from epithelial cells at final concentrations ranging from 10^{-7} to 10^{-3} M, and these samples were preincubated for 30 min at 37°. After preincubation with inhibitors, the 15,000 *g* supernatants were incubated for 15 min at 37° with 15 μM LTA_4. The reaction was quenched and the samples were extracted as previously noted [16]. Inhibitors were dissolved in ethanol or DMSO. These solvents were added to a final concentration of ≤1% and, in control experiments, had no effect on enzymatic activity.

Inhibition of aminopeptidase activity in disrupted airway epithelial cells

Aminopeptidase assays were performed in the presence or absence of the same metalloproteinase inhibitor profile as noted above for LTA_4 hydrolase assays.

Kinetic analysis of inhibition of LTA_4 hydrolase by captopril

The 15,000 *g* supernatant was diluted 1:1 in 2× assay buffer (final concentration 100 mM Tris, 0.15 M NaCl, 0.35% BSA, pH 7.8) in the presence or absence of 100 μM captopril and was prewarmed to 37° for 30 min. Concentration–response curves were then generated by incubating samples (100 μL) with 2.5 to 100 μM LTA_4 for 30 sec. Incubations were terminated abruptly by quenching with an equal volume of ice-cold methanol, and the samples were analyzed for leukotrienes. The short incubation time was used to allow accurate measures of velocity prior to the accumulation of significant quantities of product.

Inhibition of LTA_4 hydrolase activity in intact airway epithelial cells

Intact cell assays were performed using epithelial cells grown to confluence on 35-mm 6-well tissue culture plates. The cells were then incubated in HBSS containing 0.35% BSA with 15 μM LTA_4 for 15 min at 37°. The supernatants were collected, and the reaction was quenched with 1 vol. of methanol containing 100 ng of PGB_2, as an internal standard. These supernatants were then acidified to 4–4.5 with 1 M H_3PO_4 and placed at −20° for 1 hr. Precipitated proteins were removed by centrifugation at 1000 *g* at 4° for 15 min, and the supernatant was extracted with 1 vol. of chloroform. The chloroform layer was removed and evaporated, and the residue was redissolved in methanol. The samples were stored at −70° until further analysis by RP-HPLC.

Assays containing metalloproteinase inhibitors were performed by preincubating confluent cells with various concentrations of inhibitor in HBSS with 0.35% BSA for 30 min and then adding 15 μM LTA_4 for 15 min. Only inhibitors that were active in disrupted cell assays (captopril, bestatin, fosinoprilat, and 1,10-*O*-phenanthroline) were tested. The supernatants from these incubations were collected, quenched, extracted, and analyzed as per other whole cell experiments.

Effects of captopril on transcellular synthesis of LTB_4

To determine the relative ability of captopril to inhibit transcellular versus intracellular synthesis of LTB_4, stimulated synthesis of LTB_4 by neutrophils was evaluated in the presence and absence of airway epithelial cells ($9HTEo^-$). Intact neutrophils (1×10^6) were added to a confluent monolayer of airway epithelial cells in a 35-mm diameter tissue culture well and were incubated at 37° for 30 min. Cell ratios determined by direct counting of epithelial cell monolayers ranged from 0.8–3.6:1 (epithelial cells:neutrophils, respectively). Control neutrophils were incubated in a 35-mm well without epithelial cells. These samples were then preincubated for 30 min in the presence or absence of 10 μM captopril. This concentration was specifically chosen as a submaximal inhibitory concentration. All samples were then stimulated with 10 μM A23187 for 15 min at 37°. After stimulation, supernatants were collected and quenched with equal volumes of ice-cold methanol containing 100 ng PGB_2. The samples were acidified with H_3PO_4, and extracted with chloroform as previously described [12].

LTA_4 hydrolase purification and inhibition by 1,10-O-phenanthroline

The most striking difference between this study examining epithelial cells and prior studies examining neutrophils [12] is that 1,10-*O*-phenanthroline was not found to inhibit LTA_4 hydrolase significantly in epithelial cell cytosol as it has been found to inhibit LTA_4 hydrolase in neutrophil cytosol (IC_{50} = 34 μM) [12]. To determine if this difference was an intrinsic property of the epithelial cell LTA_4 hydrolase or was a function of the intracellular milieu, LTA_4 hydrolase was purified from disrupted airway epithelial cells by previously described techniques [16]. Briefly, a 100,000 *g* cytosolic fraction from epithelial cells was subjected to ammonium sulfate precipitation, and the 40–80% precipitate was then subjected to gel filtration chromatography on a 1 × 70 cm column of Sephacryl 200 HR. The column was developed with a running buffer of 100 mM Tris, pH 7.8, containing 0.02% NaN_3. Fractions (1 mL) were collected from this column and monitored for LTA_4 hydrolase activity and aminopeptidase activity. Fractions with LTA_4 hydrolase activity were subjected to DEAE cellulose anion exchange chromatography on a continuous gradient of 0 to 0.5 M NaCl using a 7.5 cm × 4.5 mm HPLC column (DEAE 5PW, Tosohaas, Montgomeryville, PA). Fractions (1 mL) were collected from the detector effluent and were monitored for LTA_4 hydrolase activity, aminopeptidase activity and total protein using the techniques described above. Fractions active for LTA_4 hydrolase activity were pooled and used to assess the effects of the metalloproteinase inhibitor, 1,10-*O*-phenanthroline.

Quantification of lipoxygenase products

Samples were dried under nitrogen and reconstituted in chromatography solvent for analysis by RP-HPLC. Leukotrienes were resolved by RP-HPLC and detected and quantitated by UV spectrophotometry and spectroscopy as previously reported [19].

Statistical analysis

All data are expressed as means ± SEM. Concentration–inhibition data were analyzed by calculating the 50% inhibitory concentration (IC_{50}) using linear interpolation. Comparisons between means were performed using an unpaired, two-tailed *t*-test. Differences were considered significant with $P < 0.05$.

RESULTS

Inhibition of LTA_4 hydrolase in disrupted airway epithelial cells

Supernatants (15,000 *g*) from disrupted $9HTEo^-$ cells were found to have significant LTA_4 hydrolase [329.9 ± 53.5 pmol LTB_4/mg protein (mean ± SD, N = 22)], as previously reported [16]. Of the inhibitors tested, only bestatin, captopril and fosinoprilat inhibited LTA_4 hydrolase activity in airway epithelial cell 15,000 *g* supernatants (Table 1). 1,10-*O*-Phenanthroline had virtually no effect on epithelial cell LTA_4 hydrolase activity (10% inhibition at 1 mM). This is in sharp contrast to prior studies in disrupted neutrophils demonstrating an IC_{50} = 34 μM for 1,10-*O*-phenanthroline with 10% inhibition occurring at concentrations of less than 0.1 μM [12]. All other inhibitors were inactive at the highest concentration tested (1 mM). Concentration–inhibition curves constructed for the active inhibitors revealed that bestatin was more potent than captopril and fosinoprilat in reducing LTA_4 hydrolase activity (IC_{50} = 7 μM, 430 μM, and 1 mM, respectively) (Fig. 1). Thus, of the angiotensin-converting enzyme inhibitors tested, only captopril and fosinoprilat had an effect on LTA_4 hydrolase activity. Quinaprilat and enalaprilat, as well as pro-forms of these drugs (quinapril and enalapril, respectively), were inactive.

Inhibition of aminopeptidase activity in disrupted airway epithelial cells

Supernatants (15,000 *g*) from disrupted $9HTEo^-$ cells were found to have significant aminopeptidase activity [1.06 ± 0.31 μmol/min/mg protein (mean ± SD, N = 21)], as previously reported [16]. Prior investigations

Table 1. Effects of metalloproteinase inhibitors on LTA_4 hydrolase and aminopeptidase activity in epithelial cell cytosol

Inhibitor	IC_{50} for hydrolase*	IC_{50} for aminopeptidase†
Amastatin	>1 mM	500 nM
Bestatin	7 μM	1 μM
Captopril	430 μM	>1 mM
Dithiothreitol	No effect	No effect
D-Penicillamine	No effect	No effect
EDTA	No effect	No effect
Enalapril	No effect	No effect
Enalaprilat	No effect	No effect
Fosinopril	No effect	No effect
Fosinoprilat	1 mM	>1 mM
1,10-*O*-Phenanthroline	>1 mM	17 μM
Phosphoramidon	No effect	No effect
Quinapril	No effect	No effect
Quinaprilat	No effect	No effect
Thiorphan	No effect	No effect

* LTA_4 hydrolase activity was measured in disrupted epithelial cells (3 mg total protein/mL) in the presence of 100 mM Tris, 0.15 M NaCl, 1 mM EDTA and 0.35% BSA, pH 7.8, for 15 min at 37°. Activity was quantitated by the measurement of LTB_4 by HPLC.

† Aminopeptidase activity was measured in disrupted epithelial cells (3 mg total protein/mL) by the cleavage of 1 mM L-alanine *p*-nitroanilide in 60 mM KPO_4 buffer, 0.15 M NaCl, pH 7.0, for 15 min at 37°. Activity was quantitated by the detection of *p*-nitroaniline release at 405 nm.

have demonstrated at least two different aminopeptidase enzymes in disrupted airway epithelial cells, and these enzymes do not have significant hydrolase activity [16]. Concentration–inhibition curves constructed for aminopeptidase activity in disrupted airway epithelial cells revealed that amastatin, bestatin and 1,10-*O*-phenanthroline were the most potent inhibitors (IC_{50} = 500 nM, 1 μM and 17 μM, respectively) (Fig. 2). None of the angiotensin-converting enzyme inhibitors was active against aminopeptidase activity even at the highest concentration tested (10 mM). These findings are consistent with the conclusion that aminopeptidase and LTA_4 hydrolase activities of airway epithelial cells are functions of different proteins.

Kinetic analysis of LTA_4 hydrolase inhibition by captopril

Kinetic analysis of LTA_4 hydrolase activity in epithelial cell cytosol revealed data that did not conform to Michaelis–Menten kinetics, as noted in earlier reports (Fig. 3) [16]. Thus, in samples not treated with captopril the data suggested two different rates of catalysis of the substrate [K_{m1} = 1.2 μM (r^2 = 0.999) and K_{m2} = 29.0 μM (r^2 = 0.982)]. In the presence of 100 μM captopril, the results conformed to Michaelis–Menten kinetics with a $K_{m\ \text{apparent}}$ of 260 μM.

Inhibition of LTA_4 hydrolase in intact airway epithelial cells

When intact airway epithelial cells were incubated with 15 μM LTA_4 for 15 min, they released 30.1 ± 3.6 pmol $LTB_4/10^6$ cells (mean ± SEM, N = 6). Only inhibitors that were active in inhibiting LTA_4 hydrolase in disrupted cells were tested in experiments using intact cells. These included bestatin, captopril, fosinoprilat, and 1,10-*O*-phenanthroline (Fig. 4). Although fosinoprilat had no significant effect, captopril, bestatin, and 1,10-*O*-phenanthroline had significant inhibitory activity in intact cell experiments (IC_{50} values of 63, 70, and 920 μM, respectively).

Effects of captopril on transcellular synthesis of LTB_4

LTB_4 synthesis was increased significantly ($P < 0.05$) by the addition of epithelial cells to A23187-stimulated neutrophils, although this increase was small when compared with prior studies [20], due to differences in cell culture conditions (Fig. 5). When neutrophils were stimulated with 10 μM A23187 in the presence of 10 μM captopril, LTB_4 synthesis was inhibited significantly 18.5 ± 4.7% ($N = 8$, $P < 0.01$) (Fig. 5). However, when neutrophils were stimulated with A23187 in the presence of epithelial cells and 10 μM captopril, synthesis of LTB_4 was inhibited 43.8 ± 2.5%. This degree of inhibition was significantly greater than the degree of inhibition observed in neutrophils alone ($P < 0.01$). These data suggest that transcellular synthesis of LTB_4 via neutrophil–epithelial cell interactions is more effectively inhibited than the direct synthesis of LTB_4 by neutrophils.

Effects of 1,10-O-phenanthroline on purified epithelial LTA_4 hydrolase

Epithelial cell cytosol was purified 179-fold by the purification scheme outlined and as previously reported [16]. The resulting specific activity of this fraction was 65.8 ± 0.4 nmol LTB_4/mg protein. When this purified cytosol was assayed in the presence of 1,10-*O*-phenanthroline, inhibition was demonstrated only at the highest concentration tested (1 mM), and the maximal inhibition observed was only 46.2 ± 14.0% (Fig. 6). These findings are in contrast to our previous work demonstrating that LTA_4 hydrolase activity in neutrophil cytosol can be inhibited by 1,10-*O*-phenanthroline with an IC_{50} value of 34 μM [12]. To further examine this issue, a concentration–inhibition curve was constructed using purified neutrophil LTA_4 hydrolase. The specific activity of control samples of the purified neutrophil preparation was 469.1 ± 21.9 nmol LTB_4/mg protein. The IC_{50} value for 1,10-*O*-phenanthroline in this neutrophil preparation was 28 μM (Fig. 6).

DISCUSSION

This study demonstrates that selected metalloproteinase inhibitors are capable of inhibiting leukotriene A_4 hydrolase in human airway epithelial cells. This inhibition is observed in spite of the fact that this enzyme in the airway seems to lack metalloproteinase (specifically aminopeptidase) activity, as has been reported previously [16]. Further, the profile of inhibitors effective against the epithelial enzyme was not identical to that of those that are active against the LTA_4 hydrolase found in the neutrophil. Thus, while 1,10-*O*-phenanthroline, a zinc-chelating agent, is an active inhibitor of neutrophil LTA_4 hydrolase (IC_{50} = 34 μM in disrupted cells) [12], this inhibitor was essentially without inhibitory activity when tested with disrupted airway epithelial cells. Captopril was also found not to inhibit the aminopeptidase activity in disrupted airway epithelial cells. This finding is consistent with prior studies [16] demonstrating that epithelial LTA_4 hydrolase, highly purified by gel filtration and anion exchange chromatography, does not have

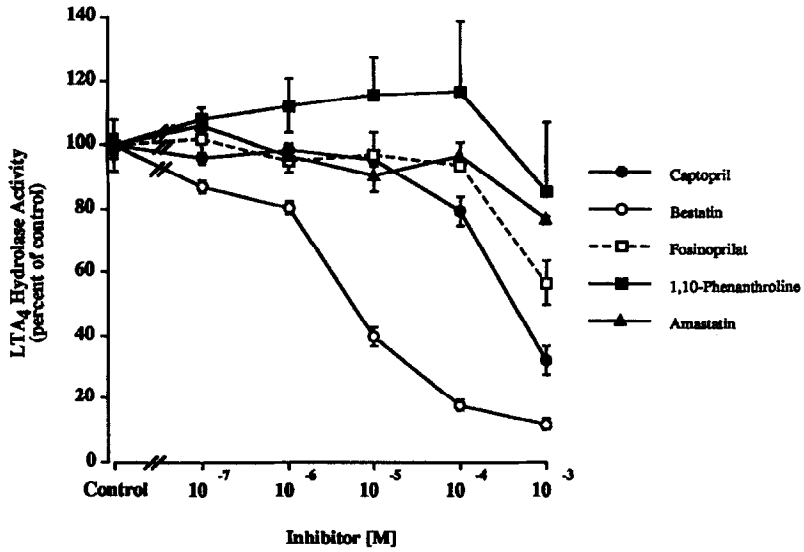

Fig. 1. Inhibition of LTA_4 hydrolase activity in disrupted epithelial cells by metalloproteinase inhibitors. Aliquots (50 μL) of 15,000 *g* supernatants from disrupted epithelial cells (3 mg total protein/mL) were incubated for 30 min at 37° in the presence of various inhibitor concentrations. Samples were then incubated with 15 μM LTA_4 for 15 min. Products were extracted and quantitated by RP-HPLC. Data are expressed as percent of control. Absolute control values were 329.9 ± 53.5 pmol LTB_4 produced/mg protein. Data represent the means ± SEM of 3 or more experiments. The IC_{50} values were as noted in Table 1.

significant aminopeptidase activity, and that epithelial cells contain other proteins with aminopeptidase activity. These data, taken together, are consistent with the hypothesis that airway epithelial LTA_4 hydrolase is a novel enzyme.

Captopril was a more potent inhibitor of LTA_4 hydrolase activity in intact airway epithelial cells than in disrupted cells. Parallel studies in erythrocytes by Orning and colleagues [10] show that captopril is a more potent inhibitor of LTA_4 hydrolase activity in intact erythrocytes than in cytosolic fractions from these cells. These observations suggest that captopril may play a role in inhibiting transcellular biosynthesis of LTB_4 by mechanisms other than direct inhibition of LTA_4 hydrolase. Consistent with these observations, studies in our laboratory have demonstrated that LTB_4 plays a role in stim-

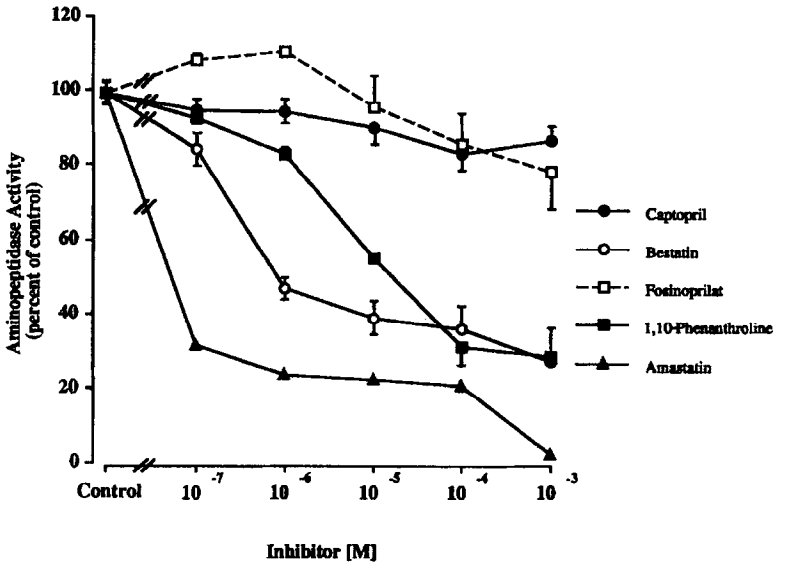

Fig. 2. Inhibition of aminopeptidase activity in disrupted epithelial cells by metalloproteinase inhibitors. Aliquots (50 μL) of 15,000 *g* supernatants from disrupted epithelial cells (3 mg total protein/mL) were incubated with 0–10 mM of various inhibitors in the presence of 1 mM L-alanine-*p*-nitroanilide for 15 min at 37°. Peptidase activity was detected by the release of *p*-nitroaniline, as measured by the change in optical density at 405 nm, and quantitated using the molar extinction coefficient (ε 9620). Data are expressed as percent of control. Absolute control values were 1.06 ± 0.31 μmol *p*-nitroaniline produced/min/mg protein. Data represent the means ± SEM of 3 or more experiments. The IC_{50} values are as noted in Table 1.

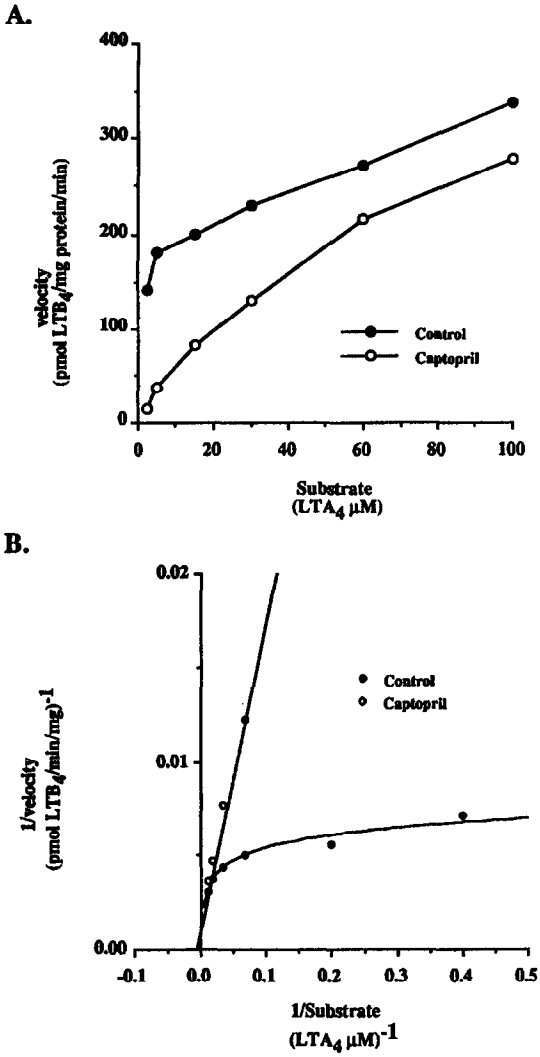

Fig. 3. Kinetic analysis of LTA_4 hydrolase inhibition by captopril in disrupted epithelial cells. Fifty microliters of 15,000 *g* supernatants from disrupted epithelial cells (3 mg/mL) was incubated with 100 μM captopril in the presence of 0–100 μM LTA_4 for 30 sec at 37°. The reaction was then quenched with ice-cold methanol, and the sample was analyzed for LTB_4 as noted in the text. Data are represented as linear plots (panel A) and a double-reciprocal plot (Lineweaver–Burk) (panel B).

ulating 5-lipoxygenase activity by a possible positive feedback mechanism [21,*]. Captopril may be a more potent inhibitor of LTB_4 synthesis in intact neutrophils by inhibiting this positive feedback effect of LTB_4. However, airway epithelial cells do not contain the enzyme 5-lipoxygenase; therefore, this feedback mechanism cannot play a role in the effects of captopril on LTB_4 synthesis in intact epithelial cells. Similarities between IC_{50} values of captopril in neutrophils and airway epithelial cells could also be the result of effects that captopril may have upon the uptake of LTA_4. Alternatively, captopril could be actively concentrated in the cell. Data from prior studies examining neutrophils are

* Serio KJ, Ring WL, Baker JR and Bigby TD, Manuscript in preparation.

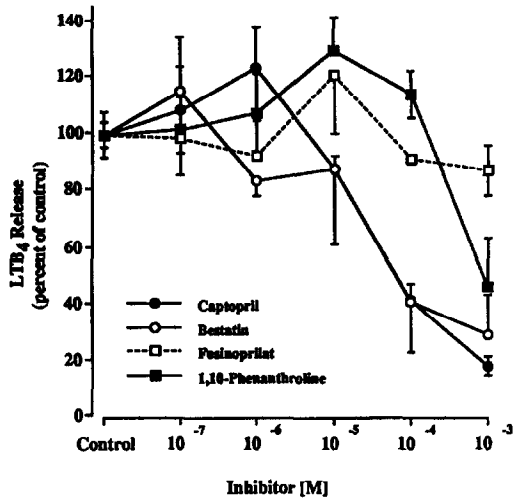

Fig. 4. Effects of metalloproteinase inhibitors on LTA_4 hydrolase activity in intact epithelial cells. Three million epithelial cells were incubated in CM-HBSS with 0.35% BSA at 37° for 30 min in the presence and absence of various concentrations of inhibitors that were active in disrupted cells. Cells were then incubated with 15 μM LTA_4 for 15 min. Products were quantitated by RP-HPLC. Data are expressed as percent of control. Absolute control values were 30.1 ± 3.6 pmol LTB_4 released/10^6 epithelial cells. Data represent the means ± SEM of 3 or more experiments.

most consistent with this latter possibility [12]. Thus, when neutrophils are incubated with captopril and subsequently washed vigorously, the inhibitory effect of captopril is not removed and persists over time [12].

The most striking difference observed in this study, when compared with prior studies examining neutrophils [10–12], is the failure of 1,10-*O*-phenanthroline to in-

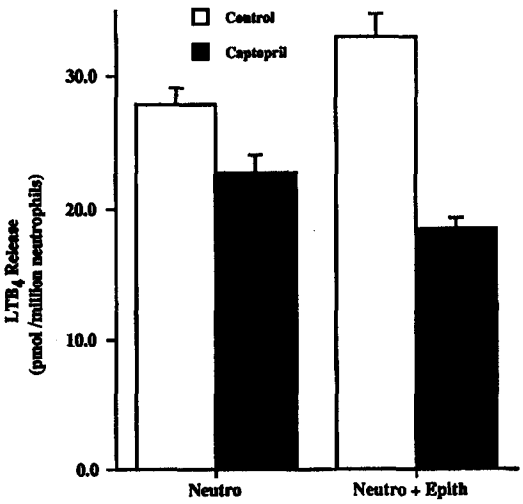

Fig. 5. Effects of captopril on the transcellular synthesis of LTB_4. Neutrophils (1×10^6) were added to confluent monolayers of epithelial cells in 35-mm diameter tissue culture wells for 30 min and then incubated for an additional 30 min in the presence or absence of 10 μM captopril. Samples were then stimulated with 10 μM A23187 for 15 min, and the supernatants were analyzed for leukotrienes. Data represent the means ± SEM of 8 experiments.

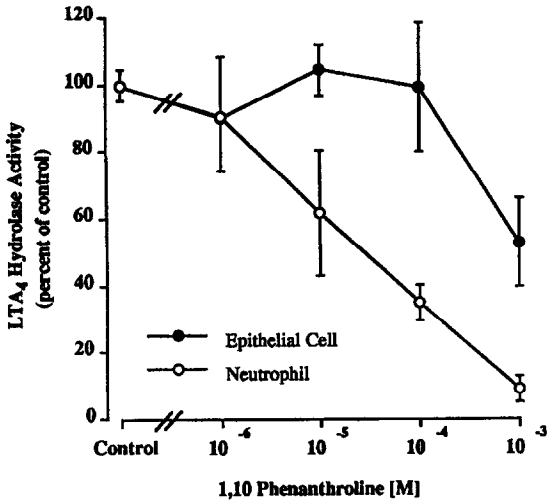

Fig. 6. 1,10-*O*-Phenanthroline concentration–inhibition curve on partially purified LTA_4 hydrolase. LTA_4 hydrolase was partially purified (179-fold) from airway epithelial cells, as described. A concentration–inhibition curve for 1,10-*O*-phenanthroline was then constructed from assays performed using this purified protein in LTA_4 hydrolase assays with concentrations of inhibitor from 10^{-6} to 10^{-3} M. For purposes of comparison, a concentration–inhibition curve was constructed using purified neutrophil LTA_4 hydrolase. Data are expressed as percent of control. Absolute control values for epithelial cell LTA_4 hydrolase were 65.8 ± 0.4 nmol LTB_4 produced/mg protein. Absolute control values for neutrophil LTA_4 hydrolase were 469.1 ± 21.9 nmol LTB_4 produced/mg protein. Data represent the means ± SEM of 3 experiments.

hibit LTA_4 hydrolase activity in epithelial cells. One potential explanation for these differences between neutrophils and epithelial cells would lie in the complex protein and cytosolic components other than LTA_4 hydrolase that are present in disrupted preparations of the two cell types. These cellular components that differ between neutrophils and epithelial cells may vary in their ability to bind or inactivate metalloproteinase inhibitors. However, 1,10-*O*-phenanthroline remained unable to inhibit LTA_4 hydrolase in epithelial cell preparations even when the enzyme was highly purified. Comparison experiments using purified neutrophil LTA_4 hydrolase revealed significant inhibition that was comparable to the inhibition observed in disrupted neutrophils. Thus, these data are most consistent with the conclusion that the LTA_4 hydrolase enzymes in epithelial cells and neutrophils are different proteins. This conclusion is also consistent with our prior studies examining airway epithelial cell [16] and neutrophil LTA_4 hydrolase activity [12].

Our data also indicate that captopril is more effective in inhibiting transcellular synthesis of LTB_4, through interactions between neutrophils and epithelial cells, than in inhibiting the direct synthesis of LTB_4 by neutrophils. The mechanism of this observed effect has not been examined by this study; however, relative differences in the uptake of captopril might explain the differential activity in the two systems. Regardless, the data suggest that a relatively weak inhibitor of LTA_4 hydrolase, such as captopril, could be more effective in inhibiting the synthesis of LTB_4 *in vivo* than would be anticipated based solely on its activity in *in vitro* systems.

The functional consequence *in vivo* of inhibition of LTA_4 hydrolase would likely be the accumulation of LTA_4. A variety of fates are possible for this excess LTA_4. LTA_4 generated by 5-lipoxygenase can be nonenzymatically hydrolyzed into biologically inactive stereoisomers of LTB_4 [22]. LTA_4, however, can serve as an intermediate for further metabolism by enzymes other than LTA_4 hydrolase. These include conversion by the enzyme LTC_4 synthase to LTC_4 [23], which can occur via a transcellular route involving platelets [20] or endothelial cells [24]. With inhibition of LTA_4 hydrolase, lipoxin formation from LTA_4 may also occur via transcellular metabolic interactions with platelets [25] and, possibly, epithelial cells. The biological consequence of the generation of LTC_4 or lipoxins during the inhibition of LTA_4 hydrolase *in vivo* is unknown, but the known biological effects of these metabolites described to date would suggest that inhibition of LTA_4 hydrolase *in vivo* could have significant physiologic effects. However, such effects have not been observed in our preliminary studies with captopril *in vivo* [12].

In summary, we have shown that metalloproteinase inhibitors are active against LTA_4 hydrolase activity in disrupted and intact human airway epithelial cells. The profile of inhibition by metalloproteinase inhibitors supports the view that LTA_4 hydrolase in human epithelial cells is a novel enzyme, distinct from the LTA_4 hydrolase present in human neutrophils. Further, captopril, a metalloproteinase inhibitor of the angiotensin-converting enzyme inhibitor type, is more effective at inhibiting LTA_4 hydrolase in intact epithelial cells than in a disrupted cell preparation, suggesting that it possesses mechanisms of action in addition to direct enzyme inhibition. Thus, captopril may be actively concentrated in the cell. Finally, captopril is more effective in inhibiting transcellular rather than direct synthesis of LTB_4 *in vitro*. Metalloproteinase inhibitors may be useful in further assessment of the *in vivo* role of LTB_4 in health and disease, and these inhibitors may also be useful in sorting out the relative contributions of different cell types to the synthesis of LTB_4.

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