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ACCELERATED COMMUNICATION

5-Lipoxygenase-Activating Protein Is the Target of a Quinoline Class of Leukotriene Synthesis Inhibitors

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SUMMARY

An indole class of leukotriene synthesis inhibitors, exemplified by MK-886, which does not directly inhibit 5-lipoxygenase, has been shown to bind to an 18-kDa leukocyte membrane protein and to inhibit 5-lipoxygenase membrane translocation. It was demonstrated that the 18-kDa protein is necessary for the cellular activation of leukotriene synthesis and was named 5-lipoxygenase-activating protein (FLAP). We describe here a class of leukotriene synthesis inhibitors based on a quinoline structure, which is structurally distinct from MK-886. However, similar to MK-886, several quinolines are potent inhibitors of cellular leukotriene synthesis but are poor inhibitors of soluble 5-lipoxygenase. To determine whether FLAP is the protein target of leukotriene synthesis inhibitors of the quinoline class, we investigated the ability of these compounds to inhibit photoaffinity labeling of FLAP and to elute FLAP from indole affinity gels. The abilities of the quinoline inhibitors to interact with FLAP correlated well with their abilities to inhibit leukotriene synthesis in human polymorphonuclear leukocytes. L-674,573, a potent quinoline leukotriene synthesis inhibitor, inhibited indole photoaffinity labeling of FLAP in a concentration-dependent manner. In addition, L-674,573 selectively eluted FLAP from indole affinity gels, in contrast to L-671,480, a quinoline that was inactive as an inhibitor of leukotriene synthesis. When human leukocyte membranes were labeled with the indole photoaffinity probe [^{125}I]L-669,083 and immunoprecipitated with a FLAP antibody, the labeling of FLAP was inhibited by L-674,573 but not by L-671,480. These results suggest a direct binding site for the quinoline leukotriene synthesis inhibitors on FLAP and provide further evidence for the essential role of FLAP in cellular leukotriene synthesis.

The diverse and potent biologic actions of the leukotrienes suggest that these mediators may play important roles in hypersensitivity and inflammatory diseases (1–3). The biosynthesis of leukotrienes is initiated by 5-lipoxygenase activation, resulting in the sequential oxidation of arachidonic acid to 5-HPETE and its dehydration to the unstable epoxide LTA_4 (4–6). LTA_4 is the precursor of the potent proinflammatory compound LTB_4 (7) and also of the peptidoleukotrienes LTC_4, LTD_4, and LTE_4, which are important mediators of bronchospasm associated with anaphylactic reactions (1–3). Although 5-lipoxygenase has been purified (8–10) and cloned (11–13), the exact mechanism of 5-lipoxygenase activation in cells is still unclear. It has been shown in human leukocytes that translocation of 5-lipoxygenase from the cytosol to a membrane site may be a critical early activation step for the enzyme (14). Members of an indole class of leukotriene synthesis inhibitor, which are active *in vivo* and in intact cells but have little effect on 5-lipoxygenase in cell-free systems, have been shown to block 5-lipoxygenase translocation (15). The protein target of

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ABBREVIATIONS: 5-HPETE, (5*S*)-hydroperoxy-6,8,11,14-eicosatetraenoic acid; FLAP, 5-lipoxygenase-activating protein; LTA_4, 5,6-oxido-7,9,11,14-eicosatetraenoic acid; LTB_4, (5*S*,12*R*)-dihydroxy-6,8,10,14-eicosatetraenoic acid; LTC_4, (5*S*)-hydroxy-(6*R*)-*S*-glutathionyl-7,9-(*trans*)-11,14-(*cis*)-eicosatetraenoic acid; LTD_4, (5*S*)-hydroxy-(6*R*)-*S*-cysteinylglycyl-7,9-(*trans*)-11,14-(*cis*)-eicosatetraenoic acid; LTE_4, (5*S*)-hydroxy-(6*R*)-*S*-cysteinyl-7-9-(*trans*)-11,14-(*cis*)-eicosatetraenoic acid; DTT, dithiothreitol; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride; PMN, polymorphonuclear leukocytes; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; MK-886, 3-[l-(*p*-chlorobenzyl)-5-isopropyl-3-*tert*-butylthioindol-2-yl]-2,2-dimethylpropanoic acid (formerly designated L-663,536); [^{125}I]L-669,083, 3-(1-(4-hydroxy-3-[^{125}I]iodophenyl)methyl)-3-(4-azidophenyl-sulfonyl)-5-isopropylindol-2-yl)-2,2-dimethyl propionic acid; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate. Buffer A, 50 mM Tris-HCl, pH 7.4, 50 μM EDTA, 20% glycerol.

this class of inhibitor, exemplified by MK-886, is a novel 18-kDa membrane protein designated FLAP, which has recently been purified from rat and human leukocytes using affinity columns based on MK-886 (16, 17). These studies showed that FLAP was selectively labeled by an indole photoaffinity probe and that the labeling was inhibited by indole analogues that inhibit leukotriene synthesis but not by indoles that do not inhibit leukotriene synthesis (16). Partial amino acid sequence obtained from the protein isolated on indole affinity columns led to the isolation of rat and human cDNAs for FLAP (18). Transfection experiments, using cDNA molecules for rat FLAP and human 5-lipoxygenase, showed that both 5-lipoxygenase and FLAP are essential for cellular leukotriene synthesis (18). Although the exact mechanism of activation of 5-lipoxygenase by FLAP remains to be clarified, it is possible that FLAP may facilitate the correct membrane localization of 5-lipoxygenase for efficient utilization of arachidonic acid. We describe here the second class of leukotriene synthesis inhibitors that interact with FLAP. We show that the affinities of the quinoline compounds for FLAP correlate with their abilities to inhibit cellular leukotriene synthesis. The ability of the quinoline compounds to elute FLAP from indole affinity gels and to compete for indole photoaffinity labeling of FLAP strongly suggests that the quinoline compounds interact with FLAP at the indole binding site.

Experimental Procedures

Rat leukocyte preparation and subcellular fractionation. Rat leukocytes (>85% PMN) were prepared from peritoneal exudates, in Eagle's minimal essential medium (GIBCO) buffered at pH 7.4 with 30 mM HEPES, 18 hr after the injection of 8 ml of 12% (w/v) sodium caseinate into 250–350-g Sprague-Dawley rats. Rat leukocytes were lysed at 1×10^8 cells/ml by sonication in 10 mM HEPES, pH 7.4, 2 mM EDTA, 1 mM β-mercaptoethanol, 1 mM PMSF, and were subjected to sequential centrifugation at 1,000, 10,000, and 100,000 × *g*, for 15, 15, and 60 min (at 4°), respectively. Photoaffinity labeling with an indole analogue of MK-886, namely [^{125}I]L-669,083, was performed as described below, using 100,000 × *g* pellet fractions that had been resuspended in buffer A and stored at −70°.

Human leukocyte preparation and subcellular fractionation. Human leukocytes were prepared from the buffy coat fraction (obtained from the Canadian Red Cross) by dextran sedimentation and hypotonic lysis of contaminating red blood cells. For preparation of the membrane fractions, leukocytes were lysed at 2×10^8 cells/ml in 50 mM KPO_4, pH 7.1, 0.1 M NaCl, 2 mM EDTA, 1 mM DTT, 0.5 mM PMSF, 60 μg/ml soybean trypsin inhibitor, and were subjected to sequential centrifugation at 10,000 and 100,000 × *g* for 15 and 60 min (at 4°), respectively. The 100,000 × *g* pellet was resuspended in 20 mM KPO_4, pH 7.1, 3 mM EDTA, 1 mM DTT, and was stored at −70° before use. Aliquots of membrane were thawed and diluted to 1 mg/ml protein in buffer A. Ammonium sulfate fractions from the 10,000 × *g* supernatant were prepared as described previously (8).

Inhibition of leukotriene synthesis in human PMN. Human PMN were isolated from whole blood that was anticoagulated with citrate (13 mM) and were purified by dextran sedimentation followed by hypotonic lysis of contaminating erythrocytes (8). Test compounds or vehicle (dimethylsulfoxide) (1 μl) were placed in 1.5-ml plastic tubes, and 0.5 ml of PMN (5×10^5 cells/ml) was added and incubated at 37° for 2 min before the addition of calcium ionophore A23187 (final concentration, 10 μM). Reactions were continued for an additional 5 min at 37° and then terminated by addition of methanol (250 μl). After centrifugation to remove precipitated proteins, 25 μl of the methanolic extract were removed for LTB_4 radioimmunoassay (19). LTB_4 in samples treated with compound was compared with that in control samples (20–30 ng/10^6 PMN), and the IC_{50} values for each compound were estimated from inhibitory dose-response curves.

Indole affinity gel elution. Rat 100,000 × *g* membrane fractions were solubilized on ice for 30 min in 50 mM Tris·HCl, pH 7.4, 140 mM NaCl, 0.5 mM DTT, 5% glycerol, 1% CHAPS. After centrifugation for 10 min at 30,000 × *g*, the supernatant was applied to 3-ml MK-886 affinity columns with ligand coupled at 60 μM (16). After washing for 16 hr at 5° with the loading buffer, absorbed proteins were eluted with 12 ml of an inactive indole or quinoline (100 μM), followed by the active indole or quinoline (100 μM) and then 0.1% SDS. Eluted samples were concentrated by vacuum dialysis overnight against 10 mM Tris·HCl, pH 7.4, 0.25 mM DTT.

[^{125}I]L-669,083 photoaffinity labeling. Membrane proteins (10–50 μg of protein) were preincubated at 37° for 2 min in Dynatech 96-well plates, in a total volume of 50 μl, in the absence or presence of test compounds dissolved in methanol (0.5 μl). [^{125}I]L-669,083 (2×10^5 cpm; 500–1000 Ci/mmol) (0.5 μl) was added, and samples were subjected to photolysis with UV light (450-W Hanovia lamp; 15 cm from sample) for 2 min at room temperature. After photolysis, 25 μl of SDS-PAGE sample buffer (190 mM Tris·HCl, pH 6.8, 37.5% glycerol, 3.75% SDS, 0.5 M β-mercaptoethanol) were added, and samples were transferred to Eppendorf tubes and boiled for 90 sec. Proteins were separated on 13.5% polyacrylamide gels by the method of Laemmli (20). Gels were dried immediately with no fixation, and the dried gels were exposed to Kodak XAR-5 film, at −70°, for 1–2 days. Molecular weights of radiolabeled bands were determined by comparison with ^{125}I marker proteins from DuPont-New England Nuclear. The intensity of the 18-kDa protein band (FLAP), relative to a nonspecifically labeled 23-kDa protein in each lane, was measured by laser densitometry using an LKB 2202 densitometer. All quantitative data relating to photoaffinity labeling were determined by densitometry.

Immunoprecipitation. Proteins from a 0–30% ammonium sulfate fraction from the 10,000 × *g* supernatant of human leukocytes (8) were preincubated, in the absence or presence of a 1 μM concentration of MK-886, L-674,573, or L-671,480, for 2 min at 37°, in 0.5 ml of 50 mM Tris·HCl, pH 7.4, 25 mM EDTA, 20% glycerol. These samples were incubated for an additional 2 min at 37°, with [^{125}I]L-669,083 (2×10^6 cpm; 500–1000 Ci/mmol). Photolysis was performed as described above, and samples were mixed with 150 μl of 1% SDS, 100 mM Tris·HCl, pH 8.0, and incubated for 15 min, with shaking, at room temperature. Samples were boiled for 5 min, followed by the addition of 0.65 ml of 2% Triton X-100, 600 mM NaCl, 20 mM Tris·HCl, pH 7.2, and incubation for 30 min at 4°. Nonspecific precipitates were removed by centrifugation at 15,000 × *g* for 15 min at 4°. To the resulting supernatant, 50 μl of FLAP antiserum H5 (a peptide antibody raised to FLAP amino acid sequence 41–52) and 4 mg of Protein A-Sepharose were added, and the mixture was incubated for an additional 60 min at 4°. The immune complex, linked to Protein A-Sepharose, was pelleted by centrifugation at 15,000 × *g* for 15 sec at 4° and was washed twice with 1% Triton X-100, 300 mM NaCl, 10 mM Tris·HCl, pH 7.2, and twice with 10 mM Tris·HCl, pH 7.0. The washed proteins were resuspended in SDS-PAGE sample buffer, boiled for 5 min, and separated on a 13.5% polyacrylamide gel by the method of Laemmli (20). The gel was dried and exposed to Kodak XAR-5 film at −70° for 48 hr. The intensity of the FLAP band was measured by densitometry.

Results

MK-886 is a potent inhibitor of the formation of cellular leukotrienes but has little direct inhibitory effect on soluble 5-lipoxygenase (15, 17, 18) (structure in Fig. 5). The novel leukocyte membrane protein FLAP has previously been shown to be the protein target of MK-886, and other leukotriene biosynthesis inhibitors of the indole class, in studies using indole affinity gels and a radioiodinated photoaffinity label based on the indole series of compounds (16). We observed that a structurally distinct class of leukotriene synthesis inhibitors, based

on the generic quinoline structure shown in Fig. 1, had properties similar to those of MK-886.

During the development of such quinoline-based leukotriene synthesis inhibitors, a large number of compounds were synthesized by our group and by others (21–23). One of the earliest quinolines to be highlighted for potential antiinflammatory therapy was REV 5901 (21). We synthesized this compound (L-656,323) and made various modifications; one modified analogue, L-655,238 (the *para*-isomer of REV 5901), was 20-fold more potent as a leukotriene synthesis inhibitor in human PMN than was REV 5901 itself (IC_{50} values of 100 nM for L-655,238 and 1.8 μM for L-656,323 or REV 5901; for structures, see legend to Fig. 3). In our initial studies, L-655,238 was used to investigate its potential affinity for FLAP, because in our early development of this quinoline class L-655,238 was our most potent leukotriene synthesis inhibitor. L-655,244, an inactive leukotriene synthesis inhibitor that is the nonsubstituted quinoline related to REV 5901, was used for comparison with L-655,238. At a concentration of 1 μM, L-655,238 and L-655,244 showed approximately 50% and no inhibition of indole photoaffinity labeling of rat or human leukocyte FLAP, respectively (Fig. 2). At a concentration of 1 μM, MK-886 inhibited photoaffinity labeling of rat and human leukocyte FLAP by approximately 90% (Fig. 2). The abilities of the compounds to compete with the photoaffinity labeling correlated with their potencies as inhibitors of leukotriene synthesis. MK-886 was 10–20-fold more potent than L-655,238 and greater than 1000-fold more potent than L-655,244 as an inhibitor of leukotriene synthesis (Fig. 3) (17). Several proteins were labeled by the photoaffinity ligand, because, when activated by UV light, its azido function is highly reactive and will attack many chemical bonds. However, in rat and human leukocyte membranes, the labeling of only the 18-kDa protein FLAP was selectively competed with by potent leukotriene synthesis inhibitors of the indole and quinoline classes. The comparison of 18-kDa protein labeling with that of the nonspecifically labeled band above it allowed for corrections for minor differences in total labeling from lane to lane. In the gel autoradiographic profiles of human leukocyte membrane proteins, the sharpness of the 18-kDa band allowed accurate densitometric calculations of inhibition of labeling by the indole and quinoline leukotriene synthesis inhibitors. Previously, we reported on the selectivity of this photoaffinity labeling, with no inhibition being shown by indole analogues of MK-886 that show no leukotriene synthesis inhibition or by direct 5-lipoxygenase inhibitors, thromboxane antagonists, dexamethasone, or disodium chromoglycate (16). In addition, a selection of prostaglandins (prostaglandins I_2, E_2, B_2, D_2, E_1, $F_{2\alpha}$, and A_1), LTC_4 LTD_4, LTB_4, arachidonic acid, and 15-hydroeicosatetraenoic acid, at 1–20 μM concentrations, were also unable to compete with the indole photoaffinity labeling of FLAP (data not shown).

More potent quinoline compounds than REV 5901 or L-655,238 were synthesized to investigate the correlation of in-

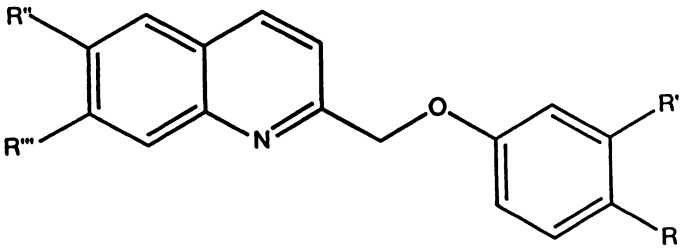

Fig. 1. Generic quinoline structure.

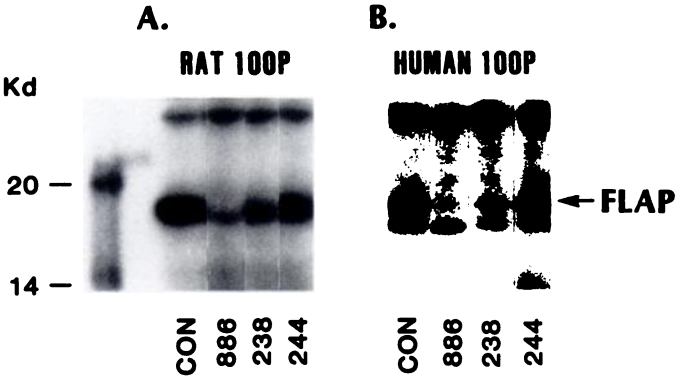

Fig. 2. [^{125}I]L-669,083 photoaffinity labeling of rat and human leukocyte membrane proteins. Autoradiograph of SDS-PAGE of proteins labeled with [^{125}I]L-669,083, as described in Experimental Procedures. *CON*, control labeling in the absence of competitor; *886*, labeling in the presence of 1 μM MK-886; *238*, labeling in the presence of 1 μM L-655,238; *244*, labeling in the presence of 1 μM L-655,244. A, Autoradiograph of labeling in rat leukocyte 100,000 × *g* pelleted membranes (*100P*). B, Autoradiograph of labeling in human leukocyte 100,000 × *g* pelleted membranes. These autoradiographs are representative of two such experiments with rat 100,000 × *g* pelleted membranes and three such experiments with human 100,000 × *g* pelleted membranes. The migration positions of ^{14}C-labeled molecular weight standards (DuPont-New England Nuclear) are shown.

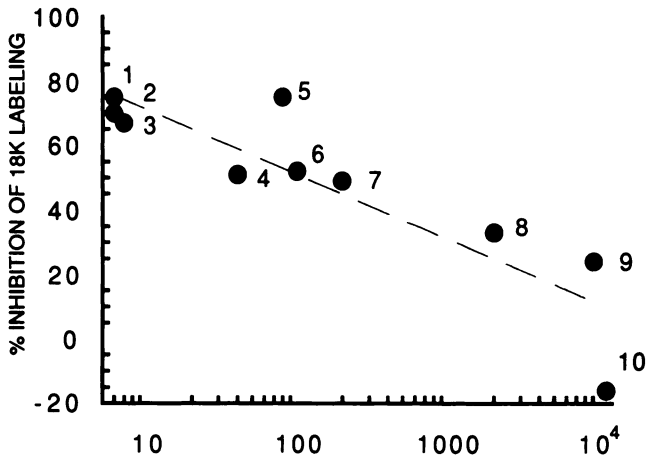

Fig. 3. Correlation of inhibition of photoaffinity labeling of human FLAP with inhibition of leukotriene (*LT*) synthesis in human PMN. Human leukocyte membrane proteins were labeled with [^{125}I]L-669,083 in the absence (control) or presence of compounds at 1 μM concentration, as described in Experimental Procedures. Results represent the average of two separate experiments in duplicate, where duplicates were ±10% and the difference between the two experiments was ±15%. The exception was compound **3**, which was only assayed in one experiment in duplicate. Inhibition of LTB_4 synthesis in human PMN was performed as described in Experimental Procedures. Results represent the average of one to six titrations. Compound **8**, L-656,323, is the Merck nomenclature for REV 5901. Structures of compounds are given by the appropriate modifications to the generic quinoline structure shown in Fig. 1. In cases where the substitution is not specified *R*, *R′*, *R″*, or *R‴* = H. **1**, L-674,573, *R* = HC(SCH_2CO_2H)[$(CH_2)_3$Ph]; **2**, L-674,574, *R* = HC[$S(CH_2)_2CO_2H$][$(CH_2)_3$Ph]; **3**, L-674,636, *R* = HC(SCH_2CO_2H)[$(CH_2)_3$-4-Cl-Ph]; **4**, L-670,592, *R* = HC(OH)[$(CH_2)_3$Ph]; **5**, L-671,447, *R* = HC(OH)[$(CH_2)_2$Ph]; **6**, L-655,238, *R* = HC(OH)[$(CH_2)_4CH_3$]; **7**, L-670,602, *R* = HC(OH)[$(CH_2)_5CF_3$]; **8**, L-656,323, *R′* = HC(OH)[$(CH_2)_4CH_3$]; **9**, L-671,480, *R* = HC(OH)[$(CH_2)_3$Ph]; *R″* = COOH; **10**, L-655,244.

hibition of FLAP photoaffinity labeling with inhibition of leukotriene synthesis, because these compounds showed only moderate to weak inhibition of leukotriene synthesis in PMN. The correlation between inhibition of indole photoaffinity labeling of human leukocyte FLAP and inhibition of leukotriene synthesis in human PMN for a series of quinolines is given in Fig. 3. Fig. 3 shows a good correlation between the percentage of inhibition of [^{125}I]L-669,083 labeling of human FLAP for 10 quinolines, at 1 μM concentration, and the logarithm of the IC_{50} for inhibition of leukotriene synthesis in human PMN ($r = -0.89$). It is not clear whether the negative value for FLAP photoaffinity labeling inhibition by L-655,244 (compound **10**) represents a real enhancement of photoaffinity labeling. One of the most potent quinoline inhibitors of leukotriene synthesis, namely L-674,573 (for structure see Fig. 5), was titrated in the FLAP photoaffinity labeling assay. A concentration-dependent inhibition of indole photoaffinity labeling of human leukocyte FLAP was observed for L-674,573, with an IC_{50} of 100 nM (Fig. 4). This IC_{50} value is similar to that observed with MK-886 in the same assay system (16). Even at high concentrations of the potent quinoline leukotriene synthesis inhibitors, such as L-674,573 (1–5 μM), 15–30% of the [^{125}I]L-669,083 labeling of FLAP was unable to be competed with (Figs. 3 and 4). In contrast, MK-886 at 1 μM showed 90–100% competition with the [^{125}I]L-669,083 labeling of human leukocyte membrane protein in the 18-kDa region (Fig. 2) (16). This may represent a small nonspecific element of the indole photoaffinity labeling that is displaced by the indole MK-886 but not by the quinoline L-674,573. MK-886 and L-674,573 had similar potencies as inhibitors of leukotriene synthesis in human PMN. At a PMN concentration of 5×10^5 cells/ml, the IC_{50} values for inhibition of LTB_4 synthesis were 3 and 6 nM for MK-886 and L-674,573, respectively (Fig. 3) (17). At a higher human PMN cell density of 4×10^7 cells/ml, the IC_{50} values for both MK-886 and L-674,573 increased to approximately 100 nM (17).[3] The reasons for the change in IC_{50} values with changes in cell density are not apparent. However, it is clear that the compounds have similar functional potencies in leukotriene synthesis inhibition assays, and these correlate with their abilities to compete with the binding of [^{125}I]L-669,083 to FLAP.

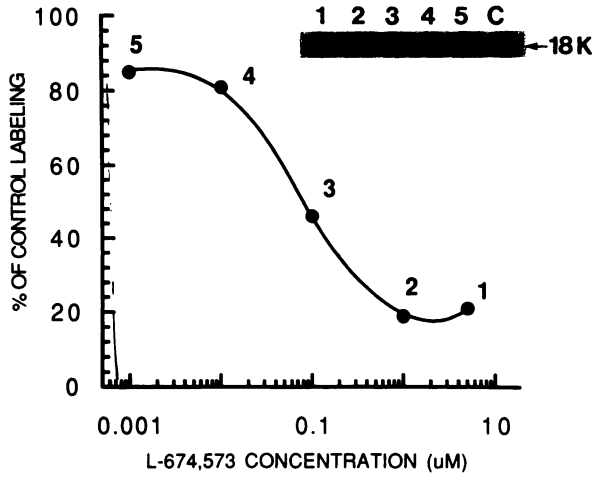

Fig. 4. Selective inhibition of indole photoaffinity labeling of human FLAP by L-674,573. [^{125}I]L-669,083 labeling of human leukocyte membrane proteins was carried out in the absence (control) or presence of various concentrations of L-674,573, as described in Experimental Procedures. Concentrations of L-674,573 were 5 μM (*point 1*), 1 μM (*point 2*), 100 nM (*point 3*), 10 nM (*point 4*), and 1 nM (*point 5*). Data are expressed as percentage of control 18-kDa protein labeling (C). The autoradiograph *inset* shows the 18-kDa region of one set of the duplicates from which the graph was derived. Each *point* is the mean of duplicate assays, where the range was not greater than ±7%. This curve is representative of two such experiments.

FLAP was originally purified on indole-based affinity gels (16). To confirm the interaction of the quinoline leukotriene synthesis inhibitors with FLAP, we compared the abilities of active and inactive indole and quinoline leukotriene synthesis inhibitors (Fig. 5) to elute rat leukocyte FLAP from such affinity gels (Fig. 6). As shown in Fig. 6, the indole compound L-583,916, which is inactive as a leukotriene synthesis inhibitor,[3] was unable to elute FLAP from the indole-based affinity matrix, in contrast to MK-886. When affinity gel to which rat membrane proteins had been prebound was washed with the relatively inactive quinoline L-671,480 (Fig. 5), FLAP remained attached to the indole-based gel. Subsequent elution with the active quinoline leukotriene synthesis inhibitor L-674,573 effectively released FLAP. Further washing of the gel with MK-886 and then SDS demonstrated that very little FLAP remained attached to the indole-based gel after elution with the quinoline L-674,573.

As a final confirmation that the interaction of the quinoline leukotriene synthesis inhibitors is with FLAP, we used an anti-FLAP peptide antiserum to immunoprecipitate [^{125}I]L-669,083-labeled human leukocyte membrane proteins (Fig. 7). This antiserum immunoprecipitated a single radioactive protein, and the labeling associated with the protein was effectively competed with by both MK-886 and L-674,573 (73 and 77% competition, respectively). In contrast, L-671,480 only showed 14% competition with the [^{125}I]L-669,083-labeled FLAP.

Discussion

A number of quinolines, including L-674,573, have been shown to be potent and specific inhibitors of leukotriene synthesis in rat and human leukocytes, but they only inhibit the activity of 5-lipoxygenase in cell-free systems at concentrations that are 100–1000-fold higher than those active in whole-cell assays (Fig. 3).[4] These observations were similar to the properties of MK-886, a member of a structurally distinct class of indole leukotriene synthesis inhibitors (17). MK-886 binds specifically to FLAP, a protein that was shown to be essential for leukotriene synthesis (16, 18). We investigated whether the leukotriene synthesis inhibitors of the quinoline class might also interact with FLAP and thereby prevent leukotriene synthesis. The abilities of quinolines to inhibit indole photoaffinity labeling of FLAP correlated well with their potencies for inhibition of leukotriene synthesis in human PMN. A similar selectivity in the abilities of quinolines to elute FLAP from indole-based affinity gels was demonstrated. L-674,573, a potent quinoline leukotriene synthesis inhibitor, effectively eluted FLAP from such affinity gels, in contrast to a relatively inactive quinoline, L-671,480. These compounds showed the same selectivity for competition with photoaffinity-labeled, immunoprecipitated FLAP, using an antibody raised to a peptide sequence obtained from cloned human FLAP (18).

The essential requirement of FLAP for the cellular synthesis

[3] S. Kargman, unpublished data.
[4] D. Riendeau, unpublished data.

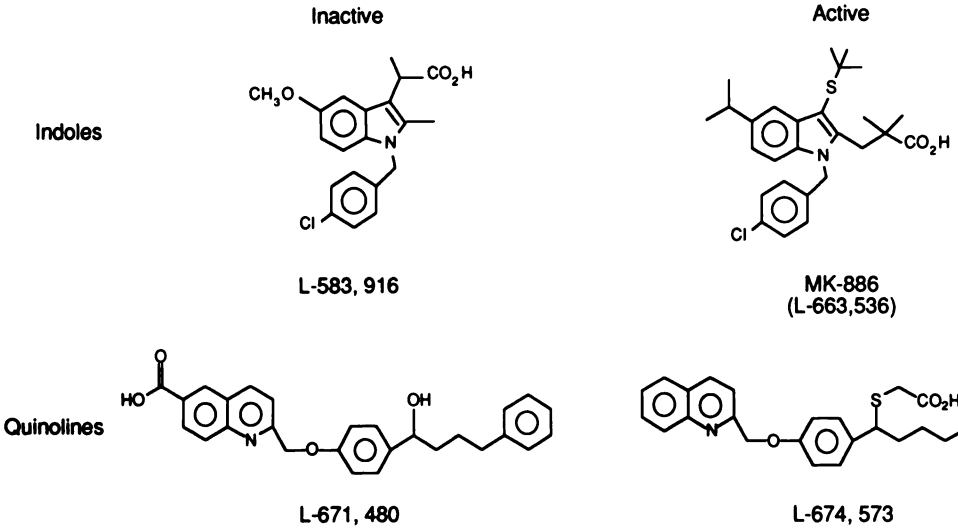

Fig. 5. Structures of leukotriene synthesis inhibitors. Compounds from both indole and quinoline classes of leukotriene synthesis inhibitors are illustrated and identified by the Merck L-number nomenclature referred to in the text.

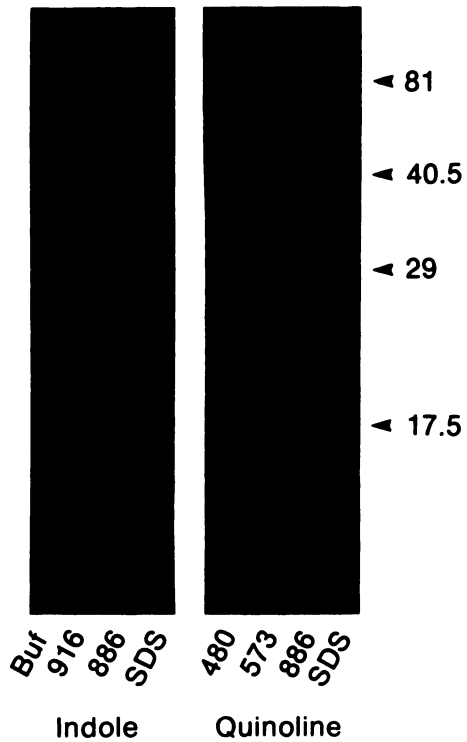

Fig. 6. Selective elution of FLAP from indole affinity gels. Elution of rat neutrophil FLAP from MK-886 affinity columns by indoles and quinolines. As described in Experimental Procedures, two identical columns were loaded with 10 mg of solubilized 100,000 × *g* membranes and washed overnight with buffer (*Buf*). One column was washed sequentially with 100 μM L-583,916 (*916*), MK-886 (*886*), and 0.1% SDS (*SDS*). The second column was washed sequentially with 100 μM L-671,480 (*480*), L-674,573 (*573*), MK-886, and SDS. Concentrated fractions were electrophoresed on 10–20% PAGE gradient gels and stained using the Daiichi silver staining method (Enprotech). The migration positions of molecular weight standards (Gelcode, Enprotech, Boston MA) are shown.

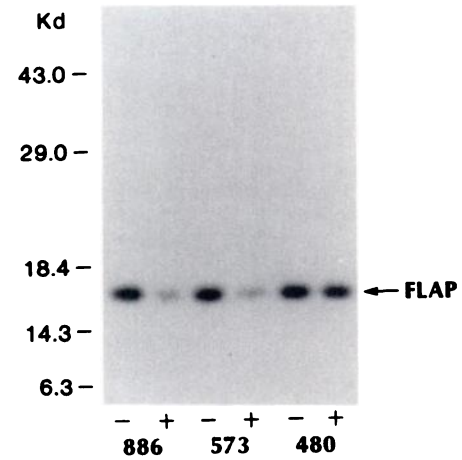

Fig. 7. Immunoprecipitation of photoaffinity-labeled FLAP. Human leukocyte proteins were labeled with [^{125}I]L-669,083 and immunoprecipitated with FLAP H5 antiserum, as described in Experimental Procedures. An autoradiograph of the immunoprecipitated samples separated by 13.5% SDS-PAGE is shown, where labeling of FLAP was performed either in the absence (–) or in the presence (+) of the following compounds at a concentration of 1 μM: *886*, MK-886; *573*, L-674,573; *480*, L-671,480. The data presented for MK-886 is representative of >50 such experiments and for L-674,573 and L-671,480 is representative of two such experiments. MK-886 is representative of >50 such experiments and competition by L-674,573 and L-671,480 is representative of two such experiments. The migration positions of ^{14}C-labeled molecular weight standards (DuPont-New England Nuclear) are shown.

of leukotrienes was confirmed here by the identification of a second class of leukotriene synthesis inhibitors that interact selectively with FLAP. The indole (MK-886) and quinoline structures are chemically quite distinct. The active moiety of either class of compound is not known. Until the ligand binding site on FLAP has been elucidated, any overlapping of functionalities of these two classes of compounds would be purely speculative. It is of interest that REV 5901, one of the earliest described leukotriene synthesis inhibitors of the quinoline class, was profiled as both a 5-lipoxygenase inhibitor and a leukotriene antagonist (21, 22). However, the 5-lipoxygenase assay reported was in fact a whole-cell system, in which the potency of REV 5901 may largely reflect interaction with FLAP, with only a weak component of direct 5-lipoxygenase inhibition. We have recently developed a FLAP radioreceptor assay, and we have shown that FLAP is the main target for indole and quinoline leukotriene synthesis inhibitors that we have synthesized and also for some of those that have been

developed by other companies, such as REV 5901 and Wyeth 49232.[5]

Although FLAP has been shown to be necessary for cellular leukotriene synthesis (18) and compounds that bind to FLAP inhibit leukotriene synthesis, the exact role of FLAP in 5-lipoxygenase activation remains to be elucidated. A key step in the synthesis of leukotrienes appears to be the translocation of 5-lipoxygenase from a cytosolic to a membrane compartment (14, 24, 25). MK-886 has been shown to inhibit and reverse membrane translocation of 5-lipoxygenase in human leukocytes (15). MK-886 has also been shown to inhibit leukotriene synthesis and FLAP photoaffinity labeling in dimethylsulfoxide-differentiated HL-60 cells (26). In recent studies, in dimethylsulfoxide-differentiated HL-60 cells, both MK-886 and L-674,573 were shown to selectively inhibit 5-lipoxygenase translocation, demonstrating that both classes of leukotriene synthesis inhibitor not only bind FLAP but also block 5-lipoxygenase membrane association.[6] The nature of the possible interaction between FLAP and 5-lipoxygenase and the way in which compounds of the indole and quinoline classes of leukotriene synthesis inhibitors prevent this association remain to be determined.

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[5]S. Charleson and J. Evans, unpublished data.
[6]S. Kargman, unpublished data.