0026-895X/92/020267-06$03.00/0

MOLECULAR PHARMACOLOGY, 41:267-272

5-Lipoxygenase-Activating Protein Is the Target of a Novel Hybrid of Two Classes of Leukotriene Biosynthesis Inhibitors

JOSEPH A. MANCINI, PETPIBOON PRASIT, MARC G. COPPOLINO, PETER CHARLESON, SERGE LEGER, JILLY F. EVANS, JOHN W. GILLARD, and PHILIP J. VICKERS

Departments of Pharmacology (J.A.M., M.G.C., J.F.E., P.J.V.), Medicinal Chemistry (P.P., S.L., J.W.G.), and Radiopharmaceuticals (P.C.), Merck Frosst Centre for Therapeutic Research, Pointe Claire-Dorval, Quebec H9R 4P8, Canada

Received September 10, 1991; Accepted November 19, 1991

SUMMARY

An 18-kDa leukocyte membrane protein, termed 5-lipoxygenase-activating protein (FLAP), has recently been shown to be the target of two structurally distinct classes of leukotriene biosynthesis inhibitors. These classes of inhibitors are based on indole and quinoline structures and are represented by MK-886 and L-674,573, respectively. A novel class of hybrid structure based on the indole and quinoline classes of inhibitors, termed quindoles, has recently been developed. These compounds, exemplified by L-689,037, are potent inhibitors of leukotriene biosynthesis, both *in vitro* and *in vivo*. In the present study, we have developed and characterized a potent radioiodinated photoaffinity analogue of L-689,037, termed [^{125}I]L-691,678. This compound was used in immunoprecipitation studies with FLAP antisera to show that the quindole series of leukotriene biosynthesis inhibitors interact directly with FLAP. In addition, we show that MK-886, L-674,573, and L-689,037 specifically compete, in a concentration-dependent manner, with both [^{125}I]L-691,678 and [^{125}I]L-669,083, a photoaffinity analogue of MK-886, for binding to FLAP. These results suggest that these three classes of leukotriene biosynthesis inhibitors share a common binding site on FLAP, providing further evidence that FLAP represents a suitable target for structurally diverse classes of leukotriene biosynthesis inhibitors.

Leukotrienes are a class of arachidonic acid metabolites synthesized by leukocytes in response to a variety of inflammatory and immunological stimuli. The potent biological actions of leukotrienes, which include the mediation of airway smooth muscle contraction and leukocyte aggregation, implicate these compounds in a number of hypersensitivity and inflammatory disorders, including asthma, psoriasis, and inflammatory bowel disease (1–5). The potential role of leukotrienes in these diseases has resulted in considerable effort to develop leukotriene biosynthesis inhibitors as potential therapeutic agents (6, 7).

The initial step in the synthesis of leukotrienes appears to be translocation of the enzyme 5-LO from a soluble to a membrane fraction (8–11). After membrane association, 5-LO catalyzes the synthesis of LTA_4 in a two-step reaction involving the oxidation of arachidonic acid to 5-HPETE, followed by the conversion of 5-HPETE to LTA_4 (12, 13). LTA_4 is subsequently converted to the proinflammatory leukotriene LTB_4 or the bronchoconstrictive peptidoleukotrienes LTC_4, LTD_4, and LTE_4, depending on the cell type (14). Because 5-LO is the key enzyme involved in leukotriene biosynthesis, most of the effort to develop leukotriene biosynthesis inhibitors has focused on the development of direct inhibitors of this enzyme (6, 7, 15). However, the clinical utility of the direct 5-LO inhibitors so far described has been limited, due to the relatively low potency and potentially toxic redox effects of the majority of such compounds (6, 16, 17).

Two classes of leukotriene biosynthesis inhibitors that do not significantly inhibit 5-LO in cell-free systems but block the cellular membrane association of the enzyme have been de-

ABBREVIATIONS: 5-LO, 5-lipoxygenase; 5-HPETE, (5*S*)-hydroperoxy-6,8,11,14-eicosatetraenoic acid; 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*)-eicosatetraeonic acid; LTE_4, (5*S*)-hydroxy-(6*R*)-*S*-cysteinyl-7,9-(*trans*)-11,14-(*cis*)-eicosatetraeonic acid; FLAP, 5-lipoxygenase-activating protein; PMN, polymorphonuclear leukocytes; MK-886, 3-[1-(*p*-chlorophenyl)-5-isopropyl-3-*tert*-butylthio-1*H*-indol-2-yl]-2,2-dimethylpropanoic acid (formerly designated L-663,536); L-689,037, ((1-(4-chlorobenzyl)-3-(3,3-dimethyl-1-oxo-1-butyl)-5-quinol-2-ylmethoxy)-indol-2-yl)-2,2-dimethyl propionic acid; L-669,083, 3-(1-(4-hydroxy-3-iodobenzyl)-3-(4-azidophenylsulfonyl)-5-isopropylindol-2-yl)-2,2-dimethylpropanoic acid; L-691,678, 3-[(4-azidophenyl)sulfonyl]-2-[(4-iodophenyl)methyl]-α,α-dimethyl-5-(2-quinolinylmethoxy)-1*H*-indole-2-propanoic acid; L-674,573, 2-((4-(1-thioacetic acid-4-phenylbutyl)phenoxy)methyl)quinoline; L-671,480, 2,2-dimethyl-8-(2-(4-(acetyloxy)tetrahydro-6-oxo-2*H*-pyran-2-yl)ethyl)-1,2,3,7,8,8A-hexahydro-3,7-dimethyl-1-naphthalenyl butanoic acid ester; L-685,079, 3-[1-(4-chlorobenzyl)-3-methoxy-5-isopropylindol-2-yl]-2,2-dimethyl propanoic acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; HPLC, high pressure liquid chromatography; IC_{50}, concentration required for 50% inhibition; DMSO, dimethyl sulfoxide.

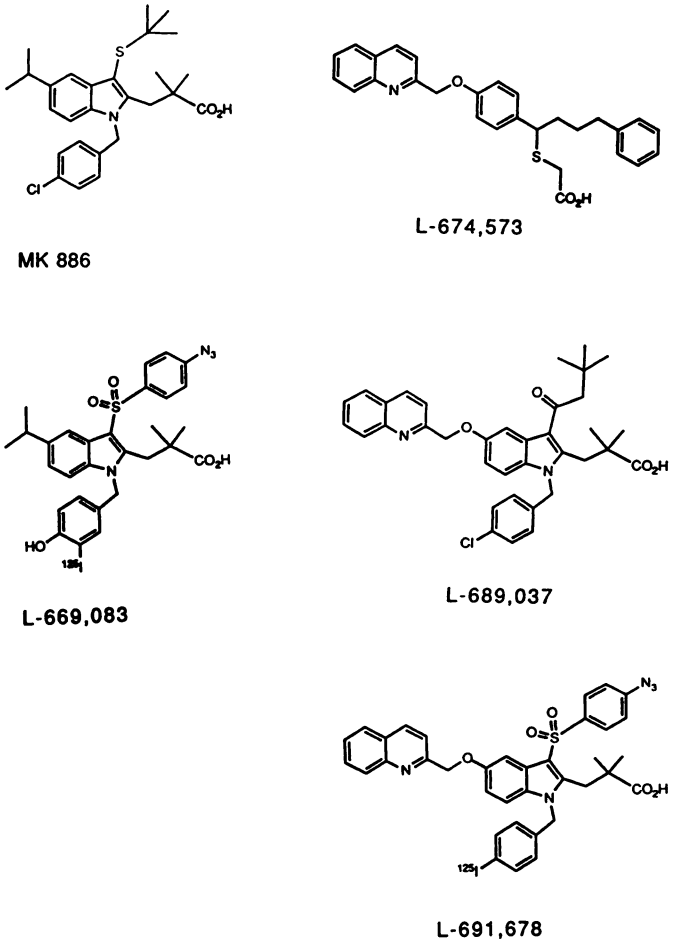

Fig. 1. Structures of leukotriene biosynthesis inhibitors.

scribed recently (18–21). MK-886 and L-674,573 (Fig. 1) represent potent members of these series of indole and quinoline compounds, respectively. The cellular target of these two classes of inhibitors appears to be an 18-kDa membrane protein termed FLAP. This protein has been purified (22) and cloned (23), and transfection experiments in osteosarcoma cells have shown that expression of both 5-LO and FLAP is essential for leukotriene synthesis (23). Recently, the gene for human FLAP has been isolated and partially characterized (24).

Although the mechanism by which FLAP activates 5-LO remains unclear, the finding that compounds that specifically bind to FLAP inhibit and reverse the membrane association of 5-LO suggests that FLAP may associate with 5-LO in a membrane complex that facilitates leukotriene biosynthesis from arachidonic acid present in membrane phospholipid. Compounds that bind to FLAP may, therefore, inhibit leukotriene synthesis by preventing the formation of such a complex.

In this study, we describe a class of hybrid molecules derived from the quinoline and indole series of leukotriene biosynthesis inhibitors (25). Members of this novel class of inhibitor, termed quindoles and exemplified by the compound L-689,037 (Fig. 1), share a similar biological profile with members of both classes of parent compounds, in that they are potent inhibitors of cellular leukotriene synthesis with no significant inhibitory effect on 5-LO activity in cell-free systems. We have developed a potent radioiodinated photoaffinity ligand based on this hybrid structure, termed [^{125}I]L-691,678, and we have used this ligand in conjunction with antipeptide antisera that recognize FLAP to show that the quindole series of leukotriene biosynthesis inhibitors interact with FLAP directly. Furthermore, competition studies using [^{125}I]L-691,678 and the indole photoaffinity ligand [^{125}I]L-669,083 suggest that the quinoline, indole, and quindole classes of compounds bind to a common site on FLAP.

Experimental Procedures

Human leukocyte preparation and subcellular fractionation. Human PMN were prepared from buffy coat concentrates by dextran sedimentation and hypotonic lysis of contaminating red blood cells, as previously described (26). The 0–30% ammonium sulfate fraction from the 10,000 × *g* supernatant of leukocyte homogenates was prepared according to previously reported procedures (26) and stored at −70° before use. This fraction has been shown to include membranes containing FLAP (22).

Synthesis and purification of [^{125}I]L-691,678. The synthesis of the methyl ester of the stannyl tributyl precursor of [^{125}I]L-691,678 will be reported elsewhere. Fifty micrograms of this compound in dimethyl formamide (60 μl) were added to 8 mCi of Na^{125}I (as a dry residue from the evaporation of an aqueous solution). The mixture was then agitated at room temperature, and 20 μg of chloramine-T were added in 10 μl of dimethyl formamide to start the iodination. After 3 min, the reaction mixture was evaporated to dryness. The radioiodinated ester was isolated by thin layer chromatography (silica gel 60 F_{254}; E. Merck number 5554; developed with hexane/ethyl acetate, 1:1) and saponified overnight at room temperature in a mixture of 150 μl of methanol and 100 μl of NaOH. The saponification mixture was acidified with citric acid, evaporated to dryness, and resuspended in water, and the solution was passed through a solid-phase extraction apparatus (Hamilton PRP-1 cartridge) to remove salts. Crude [^{125}I]L-691,678 (Fig. 1) was eluted from the cartridge with methanol.

The crude preparation of [^{125}I]L-691,678 was immediately purified by reverse phase HPLC, using a Waters HPLC system (Waters, Mississauga, Ontario, Canada). Approximately 1.5 mCi of iodinated ligand was applied to a C18 μBondapack column (Waters) and eluted with methanol/water/acetic acid (78:22:0.01), at a flow rate of 1 ml/min. The retention time of the iodinated ligand was determined by monitoring the elution of a synthetic standard of [^{127}I]L-691,678 spectrophotometrically at 291 nm (Fig. 2). After application of the radioiodinated ligand, 1-ml fractions were collected and measured for radioactivity (Fig. 2), using an LKB 1282 Compugamma counter (Pharmacia-LKB, Baie d'Urfe, Quebec, Canada). Fractions corresponding to the elution position of the iodinated ligand were pooled and stored at −70°. All solvents used were HPLC grade and purchased from BDH Chemicals (Ville St-Laurent, Quebec, Canada).

Photoaffinity labeling. The membrane fraction from human leukocytes (100 μg), resuspended in 100 μl of 50 mM Tris·HCl, pH 7.4, 25 mM EDTA, 20% glycerol, was preincubated at 37° for 2 min in Immulon 2 microtiter plates (Fisher, Montreal, Quebec, Canada), in the presence of test compounds dissolved in DMSO (1 μl). Radioiodinated photoaffinity ligand (1 μl of [^{125}I]L-669,083 or [^{125}I]L-691,678, 2 × 10^5 cpm, 500–1000 Ci/mmol) was then added, and samples were further incubated for 2 min at 37°. Samples were then exposed to a 450-W UV lamp (Hanovia, Newark, NJ), at a distance of 10 cm, for 3 min at room temperature. After UV irradiation, samples were transferred to microfuge tubes, and SDS-PAGE sample buffer was added to a final concentration of 63 mM Tris·HCl, pH 6.8, 12.5% glycerol, 1.25% SDS, 1.75 mM mercaptoethanol, 0.05% bromophenol blue. Samples were then heated at 95° for 4 min and separated on 13.5% SDS-polyacrylamide slab gels (14 cm × 12 cm × 1.5 mm) with 3% stacking gels, according to the method of Laemmli (27). Electrophoresis was performed until the dye front eluted from the gel, in order to elute unincorporated photoaffinity ligand. Gels were then fixed, dried, and exposed to Kodak

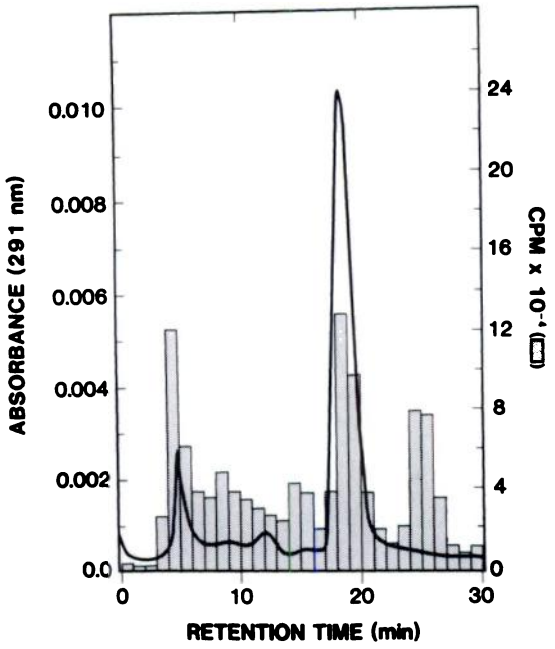

Fig. 2. HPLC purification of [^{125}I]L-691,678. [^{125}I]L-691,678 was synthesized and purified by HPLC, as described in Experimental Procedures. The elution position of a synthetic standard of [^{127}I]L-691,678 (——) is the same as the peak of radioactivity corresponding to [^{125}I]L-691,678 (fractions 18–21). Fractions corresponding to the radioiodinated ligand were pooled and stored at −70°.

XAR-2 film (Kodak, Rochester, NY) at −70° for 18–72 hr. Migration positions of proteins were compared with those of ^{14}C-labeled molecular weight standards of lysozyme (14,300), lactoglobulin (18,400), carbonic anhydrase (29,000), ovalbumin (43,000), bovine serum albumin (68,000), and phosphorylase *b* (97,400) (BRL, Burlington, Ontario, Canada). The amount of radioactivity specifically incorporated into FLAP was determined by comparing the intensity of the signal associated with FLAP with that of a nonspecifically labeled protein of approximately 25 kDa, using a model 300A scanning laser densitometer (Molecular Dynamics, Sunnyvale, CA).

Western blot analysis. The membrane fraction from human leukocytes (25 μg) was separated by 13.5% SDS-PAGE and electrophoretically transferred to nitrocellulose overnight at 100 mA, using a Transblot apparatus (Bio-Rad, Burlington, Ontario, Canada), according to the manufacturers' instructions. Western blot analysis was then performed as previously described (9), using a 1/150 dilution of rabbit polyclonal antipeptide antiserum and ^{125}I-Protein A (NEN-DuPont, Mississauga, Ontario, Canada) as the detection system. Antisera used for these studies were prepared, using standard immunization procedures, by Hazleton Research Laboratories (Denver, PA) and are designated H5 and H9. These antisera were raised to the thyroglobulin conjugates of peptides corresponding to amino acids 41–52 and 101–118 of FLAP, respectively. Both of these amino acid sequences of FLAP are identical in rat and human FLAP (23). Peptides and their thyroglobulin conjugates used for immunization were prepared by Peninsula Laboratories (Belmont, CA).

Immunoprecipitation. Immunoprecipitation of photoaffinity-labeled FLAP was performed as previously described (9).

Protein assay. Protein concentrations were determined in microtiter plates using Coomassie protein assay reagent (Pierce, Rockford, IL), according to the manufacturers' instructions, with bovine serum albumin as a standard.

Results

The indole MK-886 and the quinoline L-674,573 (Fig. 1) have recently been shown to compete specifically with [^{125}I]L-669,083 (Fig. 1), a photoaffinity analogue of MK-886, for binding to FLAP (19, 22). Moreover, within the series represented by these compounds, a correlation exists between the inhibition of photoaffinity labeling and the inhibition of leukotriene synthesis in human PMN (19, 22). A novel series of hybrid molecules containing both indole and quinoline moieties (termed quindoles) have recently been synthesized (25). L-689,037 (Fig. 1) represents a potent member of this series of inhibitors, with an IC_{50} for the inhibition of leukotriene synthesis in human PMN of 2 nM (Table 1). The pharmacological profile of this series of compounds will be described elsewhere. Similar to the indole and quinoline series of compounds (18, 19), the quindole L-689,037 does not significantly inhibit 5-LO in cell-free assays ($IC_{50} > 4$ μM).

The studies described in this paper addressed whether the inhibition of leukotriene biosynthesis displayed by the quindole series of compounds is associated with the specific binding of these compounds to FLAP. The indole [^{125}I]L-669,083 has previously been shown to specifically radiolabel FLAP in membranes from human leukocytes (22). In order to test whether the quindole series of hybrid molecules inhibit the binding of indole compounds to FLAP, membrane preparations were preincubated with the quindole L-689,037 before photoaffinity labeling by [^{125}I]L-669,083. Fig. 3 shows that L-689,037 specifically inhibits labeling of FLAP, in a concentration-dependent manner, with an IC_{50} of approximately 35 nM. The IC_{50} value of 35 nM compares with those of 80 nM and 100 nM determined

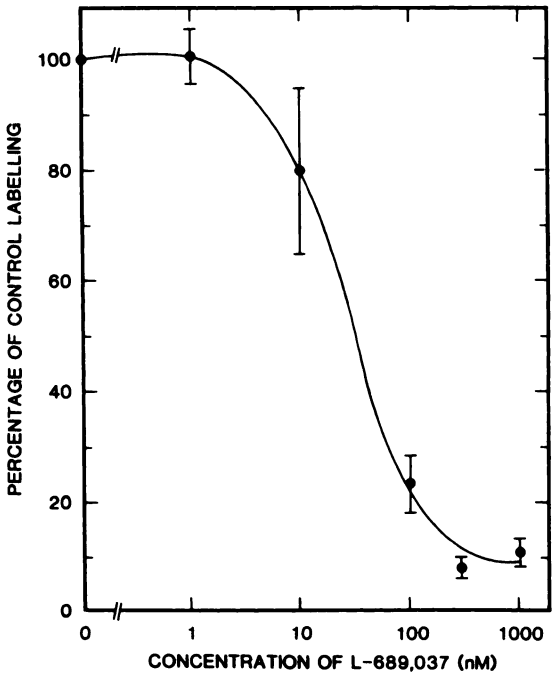

Fig. 3. Inhibition by L-689,037 of the photoaffinity labeling of FLAP by [^{125}I]L-669,083. The membrane fraction from human leukocytes was labeled with [^{125}I]L-669,083 in the presence of the indicated concentration of L-689,037 or DMSO vehicle, as described in Experimental Procedures. Samples were then separated by 13.5% SDS-PAGE, and dried gels were exposed to X-ray film for 3 days at −70°. The intensity of the band associated with FLAP, relative to a nonspecifically labeled protein of 25 kDa, was measured by scanning laser densitometry. The amount of label associated with the 18-kDa protein is expressed relative to that observed in the absence of L-689,037, and data represent the mean and range of duplicate samples.

for the inhibition of [^{125}I]L-669,083 labeling by the indole MK-886 and the quinoline L-674,573, respectively (19, 22).

To show that the quindole series of leukotriene biosynthesis inhibitors directly bind to FLAP and to further characterize the binding site of these inhibitors, we have synthesized a radioiodinated photoaffinity analogue of L-689,037, termed [^{125}I]L-691,678 (Fig. 1). Photoaffinity labeling of membrane proteins from human leukocytes was performed using this compound under conditions identical to those used for [^{125}I]L-669,083. In a similar manner to the indole [^{125}I]L-669,083 (22), the quindole [^{125}I]L-691,678 labels many membrane proteins from human leukocytes. However, the labeling of only one of those proteins, an 18-kDa protein corresponding to the migration position of FLAP, is specifically inhibited by the indole MK-886 (Fig. 4). This specific inhibition of photoaffinity labeling is concentration dependent, with an IC_{50} of approximately 30 nM (Fig. 5).

In order to assess the ability of the parent quindole and a quinoline leukotriene biosynthesis inhibitor to inhibit photoaffinity labeling by [^{125}I]L-691,678, labeling was performed after preincubation of membrane protein with various concentrations of L-689,037 and L-674,573. In a similar manner as MK-886, both L-689,037 and L-674,573 specifically inhibit labeling of an 18-kDa protein, in a concentration-dependent manner, with IC_{50} values of 45 nM and 70 nM, respectively (Fig. 5). These IC_{50} values are similar to those determined for the inhibition of [^{125}I]L-669,083 photoaffinity labeling by these compounds (Table 1). The IC_{50} values for the inhibition of photoaffinity labeling are significantly higher than the IC_{50} values for the inhibition of leukotriene synthesis in human PMN by these compounds, when measured at a cell concentration of 5×10^5/ml (20). However, in a similar manner as indole and quinoline inhibitors, the concentration of quindoles required for 50% inhibition of leukotriene synthesis varies with cell concentration and is similar to values obtained for the

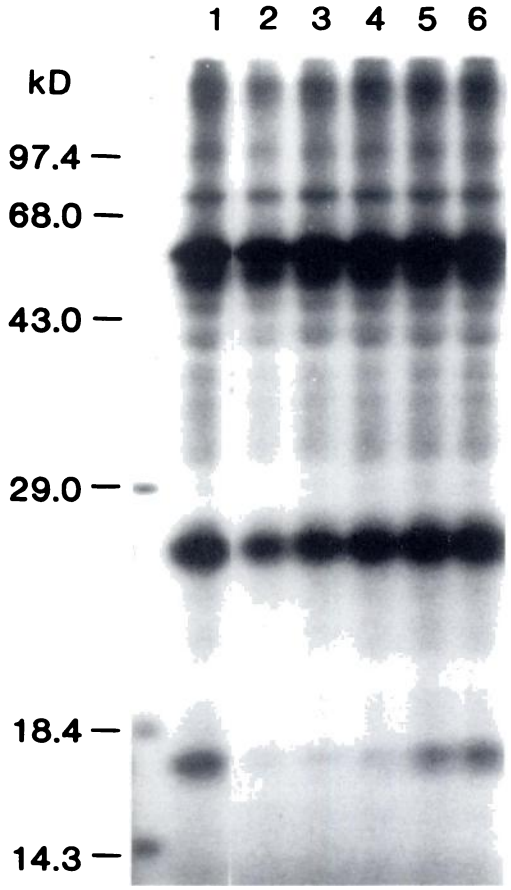

Fig. 4. Specificity of photoaffinity labeling by [^{125}I]L-691,678. The membrane fraction from human leukocytes was labeled with [^{125}I]L-691,678, and samples were separated by 13.5% SDS-PAGE, as described in Experimental Procedures. Photoaffinity labeling was performed in the presence of DMSO vehicle (*lane 1*) or MK-886 at a concentration of 1 μM (*lane 2*), 300 nM (*lane 3*), 100 nM (*lane 4*), 10 nM (*lane 5*), or 1 nM (*lane 6*). Dried gels were exposed to X-ray film for 3 days at −70°. The migration positions of ^{14}C-labeled molecular weight markers are indicated.

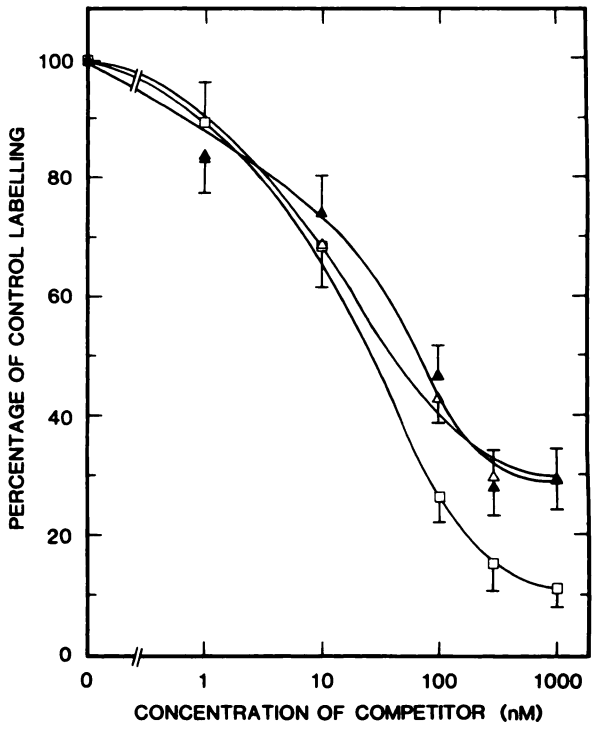

Fig. 5. Inhibition by L-674,573, L-689,037, and MK-886 of photoaffinity labeling by [^{125}I]L-691,678. The membrane fraction from human leukocytes was labeled with [^{125}I]L-691,678 in the presence of the indicated concentration of L-674,573 (▲), L-689,037 (△), or MK-886 (□), as described in Experimental Procedures. The amount of radioactivity associated with the 18-kDa protein was determined as described in Experimental Procedures.

TABLE 1

Inhibition of photoaffinity labeling of FLAP and leukotriene synthesis by leukotriene biosynthesis inhibitors

Concentrations of compounds required for the 50% inhibition (IC_{50}) of photoaffinity labeling of FLAP by [^{125}I]L-699,083 or [^{125}I]L-691,678 are indicated and compared with the IC_{50} value for the inhibition of leukotriene synthesis in human PMN at a cell concentration of 5×10^5 cells/ml.

	IC_{50}		
	[^{125}I]L-699,083	[^{125}I]L-691,678	Human PMN
		μM	
MK-886 (indole)	80[a]	30[b]	3[c]
L-674,573 (quinoline)	100[c]	70[b]	6[c]
L-689,037 (quindole)	35[b]	45[b]	2[d]

[a] From Ref. 22.
[b] Data from Fig. 5.
[c] From Ref. 19.
[d] D. Riendeau and J. Guay, unpublished result.

inhibition of photoaffinity labeling when measured at similar total protein concentrations in these assays.[1]

To confirm that the 18-kDa membrane protein that is specifically labeled with [^{125}I]L-691,678 is FLAP, a series of immunoprecipitation experiments was performed using polyclonal antisera H5 and H9, which recognize FLAP from human leukocytes (Fig. 6A). After labeling of membrane proteins from human leukocytes with [^{125}I]L-691,678, immunoprecipitation with either of these two antisera results in the labeling of a single species, corresponding to FLAP (Fig. 6B). The migration position of this protein is identical to that of the single radiolabeled species immunoprecipitated after labeling with [^{125}I]L-669,083. When photoaffinity labeling with either [^{125}I]L-669,083 or [^{125}I]L-691,678 was performed in the presence of the potent leukotriene biosynthesis inhibitor MK-886 before the immunoprecipitation procedure, essentially all of the label associated with the protein was competed. In contrast, labeling of FLAP by either photoaffinity ligand in the presence of L-685,079 or L-671,480, followed by immunoprecipitation, resulted in no significant decrease in the level of radioactivity associated with the protein. L-685,079 and L-671,480 are indole and quinoline compounds, respectively, which are only weak inhibitors of leukotriene biosynthesis in human PMN (IC_{50} values of >400 nM and 2 μM respectively).[2] Immunoprecipitation with each of the two antipeptide antisera used in these studies was inhibited by the peptide against which each antiserum was raised (Fig. 6B).

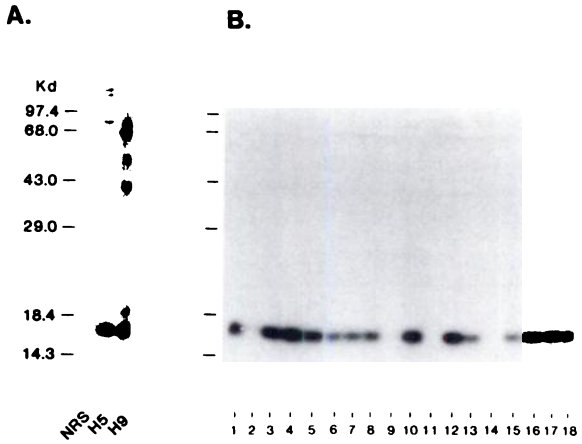

Fig. 6. Immunological detection of FLAP. A, Detection of FLAP by Western blot analysis. Western blot analysis of the membrane fraction from human leukocytes was performed as described in Experimental Procedures, using a 1/150 dilution of either normal rabbit serum (*NRS*) or antisera H5 or H9. B, Immunoprecipitation of photoaffinity-labeled FLAP. Photoaffinity labeling of the membrane fraction from human leukocytes was performed as described in Experimental Procedures, using [^{125}I]L-691,678 (*lanes 1–15*) or [^{125}I]L-669,083 (*lanes 16–18*). Labeling was performed in the presence of DMSO vehicle (*lanes 1, 3, 8, 10, 13,* and *16*), 1 μM MK-886 (*lanes 2* and *9*), 1 μM L-685,079 (*lanes 4, 6,* and *17*), 1 μM L-671,480 (*lanes 5, 7,* and *18*), or peptides (10 μg/ml) corresponding to amino acid residues 41–52 (*lanes 11* and *15*) or 101–118 (*lanes 12* and *14*) of FLAP. Immunoprecipitation was performed using H5 (*lanes 1–5, 10–12,* and *16–18*) or H9 (*lanes 6–9* and *13–15*). Immunoprecipitated proteins were separated by 13.5% SDS-PAGE, and dried gels were exposed to Kodak XAR-2 film for 4 days. The migration positions of molecular weight standards are indicated.

Discussion

These studies characterize a novel series of hybrid molecules based on quinoline and indole classes of leukotriene biosynthesis inhibitors. This new class of compounds, termed quindoles, have retained the ability of their parent compounds to act as potent inhibitors of leukotriene biosynthesis, both *in vitro* and *in vivo*, with little effect on 5-LO activity in cell-free assays. Initial experiments demonstrated that the quindole L-689,037 specifically competes with a radioiodinated photoaffinity analogue of the indole series of leukotriene biosynthesis inhibitors for binding to FLAP. This suggested that members of the quindole series of inhibitors directly interact with FLAP. In order to confirm this, a potent radioiodinated photoaffinity analogue of this series of compounds, termed [^{125}I]L-691,678, was developed. In a similar manner as for the photoaffinity analogue of the indole series (19, 22), labeling of a single human leukocyte protein by [^{125}I]L-691,678 is specifically inhibited by members of three classes of leukotriene biosynthesis inhibitors. Immunoprecipitation experiments confirmed that this 18-kDa membrane protein is FLAP and that inhibition of this labeling is only observed with compounds that are potent inhibitors of cellular leukotriene biosynthesis. These findings demonstrate that the quindole series of leukotriene biosynthesis inhibitors interact directly with FLAP, suggesting that specific binding to FLAP is the mechanism by which these compounds inhibit leukotriene biosynthesis.

When preparations of [^{125}I]L-691,678 and [^{125}I]L-669,083 of identical specific activity were compared as photoaffinity ligands, [^{125}I]L-691,678 incorporated approximately 4-fold more radioactivity into FLAP than did the indole photoaffinity ligand (data not shown). This suggests that the quindole photoaffinity ligand binds to FLAP with higher affinity than does the indole ligand. This is in agreement with the finding that the nonradioactive analogue of [^{125}I]L-691,678 is significantly more potent than the similar analogue of [^{125}I]L-669,083 at inhibiting leukotriene biosynthesis in human PMN, with IC_{50} values of 33 nM and >1.5 μM, respectively.[3] The quindole ligand, therefore, provides increased sensitivity, compared with the indole ligand, for the measurement of low levels of FLAP in membrane preparations.

The finding that indole, quinoline, and quindole inhibitors specifically compete with both indole and quindole photoaffinity ligands for binding to FLAP, and with similar IC_{50} values, strongly suggests that these three classes of compounds share a common binding site on FLAP. The availability of photoaffinity ligands from two classes of compounds that specifically interact with FLAP, several peptide antisera, and the cDNA for FLAP should allow for a determination of both the site of covalent attachment of the photoaffinity ligands and amino acid residues involved in the binding of leukotriene biosynthesis inhibitors. Establishing that members of three potent classes of leukotriene biosynthesis inhibitors specifically interact with FLAP confirms that this protein plays a key role in cellular leukotriene synthesis and may represent a suitable target for therapeutic intervention.

Acknowledgments

The authors would like to thank D. E. MacIntyre and J. Guay for PMN leukotriene synthesis inhibition assays, J.-P. Falgueyret for 5-LO inhibition

[1] J. F. Evans, unpublished observations.
[2] J. Guay and D. E. MacIntyre, unpublished observations.
[3] J. Guay, unpublished observations.

assays, J. Hutchinson for the synthesis of L-671,480, the Canadian Red Cross for human buffy coat preparations, and B. Sholzberg for secretarial services.

References

1. Samuelsson, B., S. E. Dahlen, J. A. Lindgren, C. A. Rouzer, and C. N. Serhan. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science* (*Washington D. C.*) **237**:1171–1176 (1987).
2. König, W., W. Schönfeld, M. Raulf, M. Köller, J. Knöller, J. Scheffer, and J. Brom. The neutrophil and leukotrienes: role in health and disease. *Eicosanoids* **3**:1–22 (1990).
3. Ford-Hutchinson, A. W. Evidence for the involvement of leukotrienes and other lipoxygenase products in disease states, in *Leukotrienes and Lipoxygenase* (J. Rokach, ed.). Elsevier Science Publishing, New York, 405–425 (1989).
4. Ford-Hutchinson, A. W. Leukotriene B_4 in inflammation. *Crit. Rev. Immunol* **10**:1–12 (1990).
5. Robinson, C., and S. T. Holgate. The role of leukotrienes and prostaglandins in asthma: evidence from clinical investigations. *Adv. Prostaglandin Thromboxane Leukotriene Res.* **20**:209–216 (1990).
6. Fitzsimmons, B. J., and J. Rokach. Enzyme inhibitors and leukotriene receptor antagonists, in *Leukotrienes and Lipoxygenases* (J. Rokach, ed.). Elsevier Science Publishing, New York, 427–502 (1989).
7. Cashman, J. R. Leukotriene biosynthesis inhibitors. *Pharm. Res.* **2**:253–261 (1985).
8. Rouzer, C. A., and S. Kargman. Translocation of 5-lipoxygenase to the membrane in human leukocytes challenged with ionophore A23187. *J. Biol. Chem.* **263**:10980–10988 (1988).
9. Reid, G. K., S. Kargman, P. J. Vickers, J. A. Mancini, C. Léveillé, D. Ethier, D. K. Miller, J. W. Gillard, R. A. F. Dixon, and J. F. Evans. Correlation between expression of 5-lipoxygenase-activating protein, 5-lipoxygenase, and cellular leukotriene synthesis. *J. Biol. Chem.* **265**:19818–19823 (1990).
10. Wong, A., S. M. Hwang, M. N. Cook, G. K. Hogaboom, and S. T. Crooke. Interactions of 5-lipoxygenase with membranes: studies on the association of soluble enzyme with membranes and alterations in enzyme activity. *Biochemistry* **27**:6763–6769 (1988).
11. Kargman, S., and C. A. Rouzer. Studies on the regulation, biosynthesis and activation of 5-lipoxygenase in differentiated HL-60 cells. *J. Biol. Chem.* **264**:13313–13320 (1989).
12. Shimizu, T., T. Izumi, Y. Seyama, K. Tadokor, O. Radmark, and B. Samuelsson. Characterization of leukotriene synthase from murine mast cells: evidence for its identity to arachidonate 5-lipoxygenase. *Proc. Natl. Acad. Sci. USA* **83**:4175–4179 (1986).
13. Rouzer, C. A., T. Matsumoto, and B. Samuelsson. Single protein from human leukocytes possesses 5-lipoxygenase and leukotriene A_4 synthase activities. *Proc. Natl. Acad. Sci. USA* **83**:857–861 (1986).
14. Maycock, A. L., S. S. Pong, J. F. Evans, and D. K. Miller. Biochemistry of the lipoxygenase pathways, in *Leukotrienes and Lipoxygenases* (J. Rokach, ed.). Elsevier Science Publishing, New York, 143–208 (1989).
15. Summers, J. B., B. P. Gunn, J. G. Martin, M. B. Martin, H. Mazdiyasni, A. O. Stewart, P. R. Young, J. B. Bouska, A. M. Goetze, R. D. Dyer, D. W. Brooks, and G. W. Carter. The structure-activity analysis of a class of orally active hydroxamic acid inhibitors of leukotriene biosynthesis. *J. Med. Chem.* **31**:5–7 (1988).
16. Guindon, Y., Y. Girard, A. Maycock, A. W. Ford-Hutchinson, J. G. Atkinson, P. C. Bélanger, A. Dallob, D. DeSousa, H. Dougherty, R. Egan, M. M. Goldenberg, E. Ham, R. Fortin, P. Hamel, R. Hamel, C. K. Lau, Y. Leblanc, C. S. McFarlane, H. Piechuta, M. Thérien, C. Yoakim, and J. Rokach. L-651,392: a novel, potent and selective 5-lipoxygenase inhibitor. *Adv. Prostaglandin Thromboxane Leukotriene Res.* **17**:554–557 (1987).
17. Riendeau, D., J. P. Falgueyret, D. J. Nathaniel, J. Rokach, N. Ueda, and F. Yamamoto. Sensitivity of immunoaffinity-purified porcine 5-lipoxygenase to inhibitors and activating lipid hydroperoxides. *Biochem. Pharmacol.* **38**:2313–2321 (1989).
18. Gillard, J. W., A. W. Ford-Hutchinson, C. Chan, S. Charleson, D. Denis, A. Foster, R. Fortin, S. Leger, C. S. McFarlane, H. Morton, H. Piechuta, D. Riendeau, C. A. Rouzer, J. Rokach, R. Young, D. E. MacIntyre, L. Peterson, T. Bach, G. Eiermann, S. Hopple, J. Humes, L. Hupe, S. Luell, J. Metzger, R. Meurer, D. K. Miller, E. Opas, and S. Pacholok. L-663,536 (MK-886) (3-[1-(4-chlorobenzyl)-3-*t*-butyl-thio-isopropylindol-2-yl]-2,2-dimethylpropanoic acid), a novel, orally active leukotriene biosynthesis inhibitor. *Can. J. Physiol. Pharmacol.* **67**:456–464 (1989).
19. Evans, J. F., C. Léveillé, J. A. Mancini, P. Prasit, M. Thérien, R. Zamboni, J. Y. Gauthier, R. Fortin, P. Charleson, D. E. MacIntyre, S. Luell, T. J. Bach, R. Meurer, P. J. Vickers, C. A. Rouzer, J. W. Gillard, and D. K. Miller. 5-Lipoxygenase-activating protein is the target of a quinoline class of leukotriene synthesis inhibitor. *Mol. Pharmacol.* **40**:22–27 (1991).
20. Rouzer, C. A., A. W. Ford-Hutchinson, H. E. Morton, and J. W. Gillard. MK886, a potent and specific leukotriene biosynthesis inhibitor blocks and reverses the membrane association of 5-lipoxygenase in ionophore-challenged leukocytes. *J. Biol. Chem.* **265**:1436–1442 (1990).
21. Menard, L., S. Pilote, P. H. Naccache, M. Laviolette, and P. Borgeat. Inhibitory effects of MK-886 on arachidonic acid metabolism in human phagocytes. *Br. J. Pharmacol.* **100**:15–20 (1990).
22. Miller, D. K., J. W. Gillard, P. J. Vickers, S. Sadowski, C. Léveillé, J. A. Mancini, P. Charleson, R. A. F. Dixon, A. W. Ford-Hutchinson, R. Fortin, J. Y. Gauthier, J. Rodkey, R. Rosen, C. Rouzer, I. S. Sigal, C. D. Strader, and J. F. Evans. Identification and isolation of a membrane protein necessary for leukotriene synthesis. *Nature* (*Lond.*) **343**:278–281 (1990).
23. Dixon, R. A. F., R. E. Diehl, E. Opas, E. Rands, P. J. Vickers, J. F. Evans, J. W. Gillard, and D. K. Miller. Requirement of a 5-lipoxygenase-activating protein for leukotriene synthesis. *Nature* (*Lond.*) **343**:282–284 (1990).
24. Kennedy, B. P., R. E. Diehl, Y. Boie, M. Adam, and R. A. F. Dixon. Gene characterization and promoter analysis of the human 5-lipoxygenase-activating protein. *J. Biol. Chem.* **266**:8511–8516 (1991).
25. Prasit, P., R. Fortin, J. H. Hutchinson, M. L. Belley, S. Leger, J. Gillard, and R. Frenette. (Quinolin-2-ylmethoxy) indoles as inhibitors of the biosynthesis of leukotrienes. *Can. Patent Application* **2**:023,340 (1991).
26. Rouzer, C. A., and B. Samuelsson. On the nature of the 5-lipoxygenase reaction in human leukocytes: enzyme purification and requirement for multiple stimulatory factors. *Proc. Natl. Acad. Sci. USA* **82**:6040–6044 (1985).
27. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of the bacteriophage T_4. *Nature* (*Lond.*) **227**:680–685 (1970).

Send reprint requests to: Philip J. Vickers, Ph.D., Department of Pharmacology, Merck Frosst Centre for Therapeutic Research, P.O. Box 1005, Pointe Claire-Dorval, Quebec H9R 4P8, Canada.