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SK&F 86002: A STRUCTURALLY NOVEL ANTI-INFLAMMATORY AGENT THAT INHIBITS LIPOXYGENASE- AND CYCLOOXYGENASE-MEDIATED METABOLISM OF ARACHIDONIC ACID

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Abstract—The effects of SK&F 86002 [5-(4-pyridyl)-6 (4-fluorophenyl)-2,3-dihydroimidazo (2,1-b) thiazole] on the generation of eicosanoids *in vitro* and on inflammatory responses *in vivo* are described and compared to other non-steroidal anti-inflammatory drugs. SK&F 86002 inhibited prostaglandin H_2 (PGH_2) synthase activity (IC_{50} 120 μM) as well as prostanoid production by rat basophilic leukemia (RBL-1) cells (IC_{50} 70 μM) and its sonicate (IC_{50} 100 μM) and human monocytes (IC_{50} 1 μM). In addition, SK&F 86002 inhibited the generation of dihydroxyeicosatetraenoic acid (diHETE) and 5-hydroxyeicosatetraenoic acid (5-HETE) by a high speed supernatant fraction of RBL-1 cells (IC_{50} 10 μM). Cellular production of 5-lipoxygenase products was inhibited by SK&F 86002 as measured by leukotriene B_4 (LTB_4) generation from human neutrophils (IC_{50} 20 μM), leukotriene C_4 (LTC_4) generation by human monocytes (IC_{50} 20 μM), and 5-HETE production by RBL-1 cells (IC_{50} 40 μM). The *in vivo* profile of anti-inflammatory activity of SK&F 86002 supports the dual inhibition of arachidonate metabolism as indicated by its activity in inflammation models that are insensitive to selective cyclooxygenase inhibitors. The responses of arachidonic-acid-induced edema in the mouse ear and rat paw, as well as the cell infiltration induced by carrageenan in the mouse peritoneum and by arachidonic acid in the rat air pouch, were inhibited by SK&F 86002 and phenidone but not by the selective cyclooxygenase inhibitors naproxen and indomethacin.

SK&F 86002 [5-(4-pyridyl)-6 (4-fluorophenyl)-2,3-dihydroimidazo (2,1-b)thiazole] (Fig. 1) has demonstrated anti-inflammatory and anti-arthritic activities in experimental animal models, such as carrageenan-induced rat paw edema and adjuvant arthritis [1]. These assays have been used for detecting inhibitors of prostanoid synthesis, such as the cyclooxygenase inhibitors indomethacin, naproxen and ibuprofen [2]. SK&F 86002 is effective in these models, thus indicating the *in vivo* cyclooxygenase inhibitory activity of the compound. The more recent description of the phlogistic activities of 5-lipoxygenase products [hydroxyeicosatetraenoic acids (HETES), leukotriene B_4 (LTB_4) and peptidoleukotrienes] [3, 4] and the suggestion that they participate as mediators of the humoral and cellular phases of the inflammatory response [5, 6] have stimulated a search for agents that inhibit both the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism. The rationale for this effort is strengthened by the demonstration that the anti-inflammatory activities of corticosteroids appear to be mediated via inhibition of phospholipase A_2, resulting in decreased production of both cyclooxygenase and lipoxygenase products [7, 8].

Because of the potential utility of an anti-inflammatory agent with this pharmacologic profile in the therapy of rheumatoid arthritis and other immune-mediated inflammatory diseases, the present studies examined the scope and mechanism of the anti-inflammatory activity of SK&F 86002. We report that, in addition to the inhibition of ram seminal vesicle prostaglandin H_2 (PGH_2) synthase and prostaglandin production by inflammatory cells, SK&F 86002 inhibited 5-lipoxygenase activity in extracts of RBL-1 cells and the cellular production of 5-lipoxygenase products by human polymorphonuclear leukocytes and monocytes. Furthermore, SK&F 86002 inhibited both the cellular and edematous phases of inflammatory responses which are insensitive to inhibition by selective cyclooxygenase inhibitors.

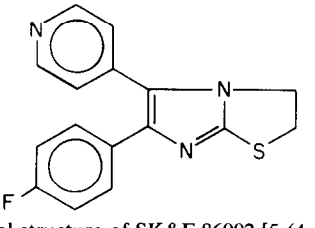

Fig. 1. Chemical structure of SK&F 86002 [5-(4-pyridyl)-6 (4-fluorophenyl)-2,3-dihydroimidazo (2,1-b) thiazole].

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MATERIALS AND METHODS

PGH_2 synthase activity. Purified ram seminal vesicle PGH_2 synthase (Biomol Research Laboratories, Inc., Philadelphia, PA) was preincubated for 90 sec in Tris–HCl buffer (pH 8.0) that contained test compound, tryptophan (5 mM) and manganese protoporphyrin (4 μM). The reaction was initiated by addition of arachidonic acid (100 μM), and oxygen consumption was monitored for 3–5 min by oxygraph recording. Typical rates using the above protocol were 0.4 to 0.6 units (μmoles O_2/min) per mg of enzyme protein. Since control rates varied between different days and over extended periods of work with a single enzyme sample, control assays were interspersed throughout each inhibitor series. Similarly, the triplicate runs of each inhibitor concentration were dispersed throughout the series of assays for a particular inhibitor.

RBL-1 5-lipoxygenase and cyclooxygenase activities. The activities of these enzymes in extracts of RBL-1 cells were assayed using the method of Jakschik and Lee [9]. RBL-1 cells were obtained from the American Type Culture Collection (no. CRL 1378) and were grown at 37° (5% CO_2 in air) in spinner culture in Eagle's Minimum Essential Medium (Gibco) (MEM) supplemented with 10% heat-inactivated fetal calf serum. Harvested cells were washed with 50 mM sodium phosphate buffer, pH 7.0, containing 1 mM EDTA and 0.1% gelatin, resuspended in fresh buffer (5×10^7 cells/ml), and disrupted by nitrogen cavitation using the Parr bomb at 750 psi for 10 min. The broken cells were then centrifuged at 10,000 *g* for 20 min. Aliquots (0.25 ml) of the supernatant fraction were preincubated with or without drugs for 10 min, and the reaction was initiated with the addition of 10 μl of $CaCl_2$ (50 mM) and 2.5 μl of 2.5 mM [1-^{14}C]arachidonic acid (final concentration was 25 μM; Amersham, Arlington Heights, IL; specific activity 20,000 dpm/nmol). After incubation for 3 min at 37°, the reaction was terminated by the addition of 2 vol. (0.5 ml) of ice-cold acetone, and the sample was allowed to deproteinize on ice for 10 min prior to centrifugation at 1000 *g* for 10 min. The deproteinized supernatant fraction was adjusted to pH 3.5 with 2 N formic acid and extracted with 2 vol. of ice-cold ethyl acetate. The efficiency of extraction of 5-HETE, diHETE and PGD_2 was 90% or better. The extracted samples were dried under argon, redissolved in ethyl acetate, and applied to Whatman LK5D thin-layer chromatography (TLC) plates which were developed using the A-9 solvent system [organic phase of ethyl acetate–2,2,5-trimethylpentane–acetic acid–water (110:50:20:10)] described by Hamberg and Samuelsson [10]. Arachidonic acid, 5-HETE, LTB_4 and PGD_2 were quantified with a Berthold LB 2832 autoscanner.

The 5-lipoxygenase and LTA_4 synthetase activities were studied further under the following conditions. An additional centrifugation of the RBL-1 supernatant fraction was carried out at 100,000 *g* for 60 min, which removes the microsomal cyclooxygenase activity. Sample incubation was done under conditions similar to those described above, i.e. 2 mM $CaCl_2$ and 25 μM [1-^{14}C]arachidonic acid (final concentration), with an incubation time of 5 min at 5°. The samples were extracted as described above and chromatographed on Whatman LK6D TLC plates that were predeveloped in the solvent system containing toluene–diethylether–ethanol–acetic acid (50:40:2:0.5). Samples were rapidly spotted and developed in the same solvent system. The radioactivity of the chromatogram was quantitated using a Berthold LB 2832 automatic TLC scanner. Under the incubation conditions, only the 5-lipoxygenase pathway metabolites were detectable. The 5-HETE and diHETEs were formed at a linear rate, and substantial amounts of the [1-^{14}C]arachidonic acid were utilized.

When assaying for inhibition of fatty acid oxygenase in intact cells, RBL-1 cells (10^7/ml) were preincubated at 37° for 5 min and then added to phosphate-buffered saline (PBS) that contained [1-^{14}C]arachidonic acid (20 μM), A23187 (10 μM), and drug in the carrier ethanol (0.5%). The incubations were stopped by adding 0.5 vol. of chilled ethanolic–formic acid (9:1) and placing the mixture on ice. The incubation mixture was extracted twice using 2 vol. of ethyl acetate. The partitioning of radioactivity in the organic extract, the aqueous phase and the emulsion interface was approximately 50, 10 and 40% respectively. The recovery of arachidonic acid, prostaglandins, 5-HETE and diHETE in the organic extract routinely was greater than 90%. The ethyl acetate phase was desiccated with Na_2SO_4 and then reduced in volume under N_2 gas. The organic extract and eicosanoid standards were applied to a Whatman LK5D TLC plate and separated with the A9 solvent system. The typical R_f values for arachidonic acid, 5-HETE, diHETE, PGD_2 and PGE_2 were 0.95, 0.70, 0.60, 0.50 and 0.30 respectively. The radioactivity on the developed plates was determined with the aid of a radioactivity autoscanner and was corrected for both detector background and auto-oxidation occurring to the precursor [^{14}C]arachidonic acid.

LTC_4 and PGE_2 production by human monocytes. Human blood monocytes were isolated according to the procedure described by Colatta *et al.* [11]. Briefly a two-stage process was employed. The blood (Red Cross, Philadelphia, PA) was initially sedimented through Histopaque (Sigma Chemical Co., St. Louis, MO) to remove red blood cells. The cells at the interface were collected, washed and sedimented through 46% Percoll (Pharmacia, Uppsala, Sweden): 54% iso-osmotic RPMI 1640 (GIBCO, Grand Island, NY). The cells at the Percoll interface were washed and used as the source of monocytes. The typical preparation consisted of 80–95% monocytes, 1–10% neutrophils, and 3–10% lymphocytes as determined by differential stain. Monocytes (3×10^6) in a volume of 0.9 ml of assay medium consisting of RPMI 1640, 25 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes), 1 mM Ca^{2+} and 5 mM Mg^{2+} were added to polypropylene tubes. Drugs were added in a volume of 0.1 ml and incubated with the cells for 45–60 min (unless otherwise indicated) at 37° in 5% CO_2 with occasional shaking. A23187 (2 μM) was added, and after a 15-min incubation, the cells were removed by centrifugation at 4°. The supernatant fractions were

stored at −70° until assayed for LTC_4 and PGE_2 levels by radioimmunoassay (RIA) (New England Nuclear, Boston, MA). Drugs were usually prepared as 10^{-2} M stocks in an appropriate organic solvent [either ethanol or dimethyl sulfoxide (DMSO)] and were further diluted in assay medium. The final concentration of organic solvent in the assays was less than 0.1%. Appropriate solvent controls were included in the experiments. The calcium ionophore A23187 (Sigma) was also prepared as a 10^{-2} M solution in DMSO and further diluted to 2 μM with assay medium.

LTB_4 production from human neutrophils. Neutrophils were isolated from whole blood by passage through Ficoll/Hypague. These cells (5×10^6) were preincubated in 1.975 ml of PBS with test compound for 15 min at 37°. A23187 (1 μM; 25 μl) was added to stimulate LTB_4 production. After 5 min of incubation with ionophore, PGB_2 (2 nmol) was added as an internal standard, and the samples were acidified with 2.4 ml of 10% acetic acid and placed on ice. LTB_4 and its oxidation products were extracted from the supernatant fraction using C_{18} (3 ml) cartridges (J. T. Baker Chemical Co., Phillipsburg, NJ). Columns were preconditioned according to the manufacturer's recommendation. After addition of the samples to the cartridges, they were eluted in the following order with water (6 ml), 10% ethanol (2 ml), petroleum ether (2 ml), and finally with 2 ml of methyl formate. The methyl formate fraction was dried under nitrogen and resuspended in 100 μl of 30% acetonitrile in 50 mM ammonium acetate just prior to quantitation by HPLC.

6-*trans*-LTB_4, LTB_4 and its oxidized metabolites, 20-hydroxy-LTB_4 and 20-carboxy-LTB_4, were separated on an HPLC system consisting of two Waters model 510 solvent delivery systems, a model 840 control station, a model 481 variable wavelength u.v. detector set at 280 nm, a model 710 autoinjector and a model RCM-100 radial compression module that contained a NOVA PAK (8×100 mm) C_{18} column (Waters Associates, Inc., Milford, MA). Separation was obtained with a flow rate of 2.5 ml/min and by gradient elution varying the amounts of water and acetonitrile, both of which were acidified with 1% acetic acid. The percentage of acetonitrile was increased linearly from 32 to 33% over 10 min, then to 50% over 2 min, then increased linearly to 56% over 6 min, and then linearly to 90% over 3 min, and held for an additional 3 min before re-equilibration. Compounds were identified by comparison of HPLC elution time to synthetic standards (Biomol Research Laboratories, Inc.). The amount of 6-*trans*-LTB_4, LTB_4, 20-hydroxy-LTB_4 and 20-carboxy-LTB_4 were quantitated from standard curves of synthetic standards and PGB_2 as an internal standard.

Animals. Male Balb/c mice and male Lewis rats were obtained from Charles River Breeding Laboratories (Kingston, NY). Within a single experiment mice (20–28 g) and rats (140–240 g) were age-matched.

Mouse carrageenan peritonitis. Mice were pretreated with either the test compound or vehicle (administered orally) 1 hr before the intraperitoneal injection of a 1.0% carrageenan (Viscarin, Marine Colloids, Springfield, NJ) suspension in saline (0.2 ml/mouse). Mice were killed by cervical dislocation 2 hr after injection, and 3.0 ml of PBS, without Ca^{2+} or Mg^{2+}, was injected into the peritoneum. Following massage, a 2.0-ml aliquot of the lavage fluid was removed, and the total cell count determined on a Coulter counter (Coulter Electronics, Hialeah, FL) and the differential cell count determined by microscopic counting of Giemsa-stained slides. Resident populations or saline-injected mice had less than 1% neutrophils.

Arachidonic-acid-induced mouse ear inflammation. Arachidonic acid (Sigma Chemical Co.) in acetone (2 mg/20 μl) was applied to the inner surface of the left ear. The thickness of both ears was then measured with a dial micrometer (Mitutoyo, Japan) 1 hr after treatment, and the data were expressed as the change in thickness (10^{-3} cm) between treated and untreated ears. The application of acetone did not cause an edematous response; therefore, the difference in ear thickness represented the response to arachidonic acid.

Test compounds were given orally in 0.5% tragacanth (10 ml/kg) at the indicated times prior to the topical application of arachidonic acid.

Arachidonic-acid-induced rat paw swelling. Paw edema was induced by a single subplantar injection of 0.10 ml arachidonic acid (0.5%) in 0.2 M carbonate buffer (pH 8.5) into the rats' right hindpaw. Drug or vehicle was administered orally in aqueous 0.5% tragacanth in a volume of 10 ml/kg either 0.5 or 2 hr prior to the arachidonic acid injection. Hind paw edema was measured plethysmographically [12], and defined as the difference in hind paw volume prior to and after arachidonic acid injection. The paw edema response to arachidonic acid averaged 0.7 ml (± 0.1), whereas the carbonate buffer, itself, provoked only a 0.02 ml (± 0.02) increase in paw edema.

Arachidonic-acid-induced air pouch inflammation. Rats were shaved on the dorsal flank and then injected subcutaneously 1 day later with 20 ml of air to form a defined pouch [13]. The air pouch was reinflated as necessary over the next 6 days. To assay anti-inflammatory activity, animals were treated orally with test compound or vehicle (10 ml/kg) 2 hr before injection of 5 ml of 0.1% arachidonic acid in 0.2 M bicarbonate buffer. Animals were killed using CO_2 3 hr after instillation of irritant. The exudate was aspirated from the pouch, and neutrophil count and differential cell count were measured. Bicarbonate buffer injection alone resulted in neutrophil infiltration that averaged 25% of that seen with arachidonic acid.

Reagents. SK&F 86002, phenidone, indomethacin, naproxen, and ibuprofen were each used as the free base. SK&F 86002 was obtained from the Drug Substances and Products Registry of Smith Kline & French Laboratories, and all other pharmacological reagents were obtained from the Sigma Chemical Co. For *in vivo* administration, the compounds were homogenized in 0.5% tragacanth. Compounds were administered by gavage at the indicated dose in a final volume of 10 ml/kg.

For *in vitro* experiments, compounds were dissolved at appropriate concentrations in ethanol or DMSO and then diluted to final concentrations using

the buffers indicated in the text (final concentration <0.1%).

Statistics and data analysis. Statistical analysis was done using Student's *t*-test.

The inhibitory concentration that caused a 50% inhibition (IC_{50}) was calculated by regression analysis of the dose–response data. The ED_{25} and ED_{50} are values which caused a 25% and 50% (respectively) inhibition of the inflammatory response *in vivo* and were also calculated by regression analysis of the dose–response data.

RESULTS

Inhibition of cell-free cyclooxygenase and 5-lipoxygenase activities by SK&F 86002. As a first step in assessing the anti-inflammatory actions of SK&F 86002, we addressed the inhibitory actions on eicosanoid production using various cell-free preparations of the fatty acid oxygenases. Our studies on cyclooxygenase were performed with PGH synthase purified from ram seminal vesicle. The cyclooxygenase activity was continually monitored by oxygen consumption, and drug action on the velocity of the enzyme was determined. SK&F 86002 proved to be a relatively weak inhibitor of cyclooxygenase activity having an IC_{50} of 120 μM as compared to indomethacin, a time-dependent, irreversible inhibitor, having an IC_{50} of 1 μM (Table 1). Inhibition of 5-lipoxygenase activity was assessed using the 100,000 *g* supernatant fraction of RBL-1 cells. This preparation expressed only 5-lipoxygenase/LTA_4 synthase activity, as indicated by the generation of 5-HETE and 5,12-diHETE from [^{14}C]arachidonic acid. No prostanoid products were detected in this preparation. SK&F 86002 inhibited the generation of 5,12-diHETE and 5-HETE in a dose-related fashion with an IC_{50} of 10 μM (Table 1). Repeat experiments using various enzyme preparations yielded a range of IC_{50} values from 7 to 30 μM. Greater than 90% inhibition was obtained at 100 μM SK&F 86002. Phenidone (IC_{50} 5 μM) was more potent than SK&F 86002 in inhibiting 5-lipoxygenase activity (Table 1).

To determine the relative potency of SK&F 86002 in inhibiting both cyclooxygenase and 5-lipoxygenase in the same cell-free system, we used the RBL-1 sonicate to which 100 μM arachidonic acid was added. SK&F 86002 inhibited the production of both the cyclooxygenase product PGD_2 (IC_{50} 100 μM) and the 5-lipoxygenase product 5,12-diHETE (IC_{50} 75 μM) (Table 1). Although more potent than SK&F 86002, phenidone acted as a relatively selective 5-lipoxygenase inhibitor (IC_{50} 10 μM) and indomethacin acted as a selective cyclooxygenase inhibitor (IC_{50} 2.5 μM).

Inhibition of cellular eicosanoid production by SK&F 86002. Eicosanoid production in intact cells was studied in order to determine the influence of the cellular environment on the inhibitory properties of SK&F 86002. Neutrophils and monocytes were isolated from human peripheral blood. On stimulation with A23187 (1 μM), neutrophils produced LTB_4 and its isomers 6-*trans*-LTB_4 and 6-*trans*-12epi-LTB_4. SK&F 86002 and phenidone inhibited the generation of these 5-lipoxygenase products with IC_{50} values of 20 and 5 μM respectively (Fig. 2A). The ratio of LTB_4 and its isomers (2:1) was not affected by drug treatment. Stimulation of monocytes with A23187 (2 μM) induced LTC_4 and PGE_2 production which were quantitated by RIA. In this system, SK&F 86002 inhibited LTC_4 (Fig. 2B) and PGE_2 (Fig. 3A) production with IC_{50} values of 20 and 1 μM respectively. In contrast, phenidone inhibited LTC_4 production (IC_{50} 7 μM) (Fig. 2B) and PGE_2 production (IC_{50} ~ 10 μM, data not shown).

RBL-1 cells expressed both cyclooxygenase and lipoxygenase activities in approximately equal amounts and, when the cells were cultured in suspension, required exogenous arachidonic acid to express their oxygenase activities. In the presence of 20 μM [^{14}C]arachidonic acid and 10 μM A23187, optimal activities of both oxygenases were expressed with 20% of the added substrate being converted to eicosanoids. Incubation with SK&F 86002 caused a dose-dependent inhibition of both activities with IC_{50} values of 40 μM for 5-HETE inhibition (Fig. 2C) and

Table 1. Inhibition of cyclooxygenase and 5-lipoxygenase activities

	Cyclooxygenase		5-Lipoxygenase	
	IC_{50} (μM)		IC_{50} (μM)	
Compound	Enzyme*	Sonicate†	Enzyme‡	Sonicate‡
SK&F 86002	120	100	10	75
Indomethacin	1	2.5	200	Inactive at 30
Phenidone	NT§	Inactive at 100	5	10

* Test compounds were added to cyclooxygenase 90 sec before the addition of 100 μM arachidonic acid. The resulting oxygen consumption was recorded, and the inhibition of optimal velocity calculated.

† Sonicate activities were measured by the production of PGD_2 and diHETE from added [^{14}C]arachidonic acid incubated with a sonicate of RBL-1 cells.

‡ High speed supernatant fractions from RBL-1 cells were added to an incubation mixture of [^{14}C]arachidonic acid (25 μM) and the test compound (see Materials and Methods for details). The results represent mean values derived from measurements on four replicate analyses.

§ Not tested.

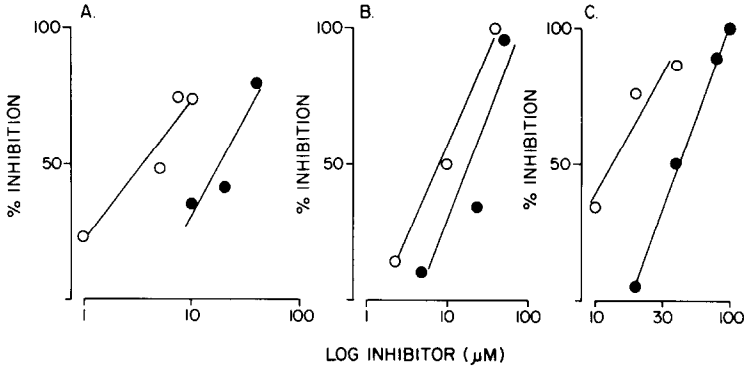

Fig. 2. Inhibition of 5-lipoxygenase activity in different cell types. 5-Lipoxygenase activity was measured by quantitating product formation. (A) Human neutrophils were treated with SK&F 86002 (●) or phenidone (○) at the indicated concentrations and challenged with 1 μM A23187 for 5 min. LTB_4 and its isomers were quantitated by reverse phase HPLC, and the results reported as the means for four experiments. (B) Human monocytes were treated with either SK&F 86002 (●) or phenidone (○) at the indicated concentrations and immediately challenged with 2 μM A23187 for 15 min. LTC_4 production was measured by RIA, and the results are representative of three experiments. (C) RBL-1 cells were treated with either SK&F 86002 (●) or phenidone (○) and immediately challenged with 20 μM [^{14}C]arachidonic acid and 10 μM A23187 for 5 min. [^{14}C]5-HETE was extracted, separated by TLC, and identified by cochromatography with standard. The results are the mean for three experiments. The SD for each point was less than 15% of the corresponding mean.

70 μM for PGD_2 inhibition (Fig. 3B). Under the same conditions, phenidone was a more potent inhibitor of lipoxygenase activity with an IC_{50} of 15 μM, and ibuprofen inhibited cyclooxygenase with an IC_{50} of 20 μM.

Effects of SK&F 86002 on leukocyte infiltration into inflammatory lesions. To determine that dual inhibition of eicosanoid metabolism would result in an effective anti-inflammatory action, SK&F 86002 was evaluated in *in vivo* models of cell infiltration into inflammatory lesions which are insensitive to the action of selective cyclooxygenase inhibitors. As shown in Table 2, the infiltration of neutrophils induced by intraperitoneal injection of carrageenan in mice was reduced dramatically by oral administration of SK&F 86002. The inhibition was dose related, with an ED_{50} of 40 mg/kg, p.o. The lipoxygenase inhibitor phenidone was also active in this peritonitis model (ED_{50} = 70 mg/kg, p.o.). In contrast, the cyclooxygenase inhibitors indomethacin (10 mg/kg, p.o.) and naproxen (100 mg/kg, p.o.) did not inhibit the cell infiltration in this assay system despite their administration at near maximally tolerated doses (Table 2). Inhibition of cell infiltration induced by arachidonic acid injection into the rat air pouch model was examined. As shown in Table 3, SK&F 86002 and phenidone significantly reduced neutrophil and mononuclear cell influx, whereas indomethacin was inactive.

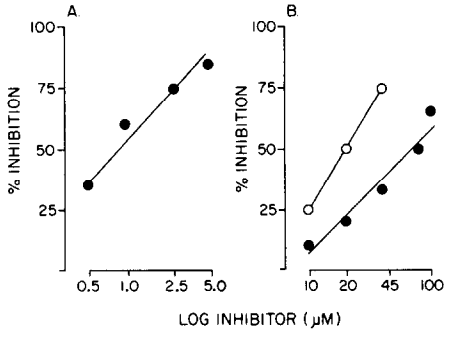

Fig. 3. Inhibition of cyclooxygenase activity in different cell types. Cyclooxygenase activity was recorded in the same experiments as the lipoxygenase activity measurements in Fig. 2 and was quantified by measuring prostaglandin production. (A) human monocytes were treated with SK&F 86002 at the indicated concentrations and immediately challenged with 2 μM A23187 for 15 min. PGE_2 production was measured by RIA. (B) RBL-1 cells were treated with either SK&F 86002 (●) or ibuprofen (○) and then challenged by 20 μM [^{14}C]arachidonic acid and 10 μM A23187. PGD_2 was extracted, separated by TLC, and identified by cochromatography with standard. The results are the mean for three experiments. The SD for each point was less than 15% of the corresponding mean.

Effect of SK&F 86002 on arachidonic-acid-induced edema. The anti-inflammatory activity of SK&F 86002 was evaluated further using arachidonic-acid-induced edema in the mouse ear and in the rat paw. The edematous response to arachidonic acid applied to the mouse ear has been shown previously to be sensitive to agents that inhibit 5-lipoxygenase products of arachidonate metabolism [14]. Oral administration of SK&F 86002 (ED_{50} 27 mg/kg, p.o.) and phenidone (ED_{50} 38 mg/kg, p.o.) produced marked inhibition of the ear edematous response induced by arachidonic acid application (Table 4). In contrast, the cyclooxygenase inhibitors indomethacin (10 mg/kg, p.o.), ibuprofen (250 mg/kg, p.o.) and naproxen (100 mg/kg, p.o.), administered at near maximally tolerated doses, did not exhibit detectable anti-inflammatory activity in this assay. Notably, at high doses, significant stimulation was observed with ibuprofen treatment (Table 4).

Arachidonic-acid-induced edema in rat paw was a second *in vivo* model used to discriminate between

Table 2. Effect of SK&F 86002, phenidone, indomethacin and naproxen on the infiltration of neutrophils into sites of carrageenan-induced inflammation

Treatment*	Dose (mg/kg, p.o.)	Neutrophils × 10^5/ml	% Change
SK&F 86002	100	2.5 ± 1.0†	−77
	50	4.8 ± 3.0‡	−56
	25	7.9 ± 4.1	−27
Phenidone	200	0.7 ± 0.5†	−94
	100	7.0 ± 3.8§	−36
	50	5.3 ± 2.2‡	−51
Indomethacin	10	8.2 ± 2.7‖	−12
Naproxen	100	10.3 ± 2.5‖	−11

* Mice were pretreated orally with the compounds indicated 1 hr prior to the i.p. injection of carrageenan, and cellular infiltration was measured 2 hr later as described in Materials and Methods. The data represent mean ± SD values derived from measurements on five animals in each treatment group. Control values for these experiments ranged from 7.8 ± 2.6 to 11.5 ± 3.7 neutrophils × 10^{-5}/ml.

† Statistically significant at $P < 0.001$.

‡ Statistically significant at $P < 0.01$.

§ Statistically significant $P < 0.05$.

‖ Not significant.

Table 3. Effect of SK&F 86002, phenidone and indomethacin on arachidonic-acid-induced cellular infiltration into the rat "air pouch"*

Treatment	Cellular infiltrate (total × 10^6)	
	Neutrophil	Monocytes
Control	4.4 ± 3.7	9.8 ± 4.7
SK&F 86002 (100 mg/kg)	1.2 ± 0.7†	1.5 ± 1.1‡
Phenidone (100 mg/kg)	1.3 ± 0.8†	2.2 ± 1.5‡
Indomethacin (5 mg/kg)	5.0 ± 3.8	7.0 ± 6.4

* Test compound or vehicle was administered orally 2 hr prior to injection of arachidonic acid. Cell infiltration was measured 3 hr after injection of arachidonic acid into a preformed air pouch as described in Materials and Methods. The results represent mean values (±SD) derived from measurement of six to eight animals.

† Statistically significant at $P < 0.05$.

‡ Statistically significant at $P < 0.01$.

lipoxygenase inhibitors and selective cyclooxygenase inhibitors [15]. Neither ibuprofen nor indomethacin blocked the edema formation (Table 5). When administered orally, phenidone inhibited the edema formation in a dose–response fashion with an ED_{25} of 25 mg/kg. SK&F 86002 (ED_{25} 15 mg/kg) was a more effective inhibitor of the edematous response showing 50% inhibition at a dose of 40 mg/kg.

DISCUSSION

Investigation of the role of arachidonic acid metabolites in immune- and non-immune-mediated inflammation in different organs is a major area of inquiry [4, 16, 17]. In addition to the well-documented phlogistic activity of the prostaglandins [18], the more recent detection of similar activities in the leukotrienes has broadened interest in these products as mediators of inflammation and as targets for pharmacologic inhibition of both the fluid and cellular phases of inflammation. Non-steroidal anti-inflammatory agents such as indomethacin, naproxen and

Table 4. Effect of SK&F 86002, phenidone and selective cyclooxygenase inhibitors on arachidonic-acid-induced inflammation of the mouse ear*

Treatment	Dose (mg/kg, p.o.)	Increase in ear thickness at 1 hr (× 10^{-3} cm)	% Change
SK&F 86002	50	6.4 ± 0.6†	−70
	25	11.8 ± 2.0†	−45
	12.5	17.6 ± 0.8‡	−18
Phenidone	100	10.2 ± 1.8†	−63
	50	10.6 ± 2.1†	−61
	25	18.8 ± 1.4†	−32
Indomethacin	10	24.4 ± 0.8	−5
Naproxen	100	26.4 ± 2.6	+3
Ibuprofen	250	30.8 ± 2.0	+20

* Compounds were administered 15 min before application of arachidonic acid to the ear as described in Materials and Methods. The results represent mean values ± SE derived from measurements on five animals. Control values for these experiments ranged from 21.4 ± 1.1 to 30.0 ± 1.3

† Statistically significant at $P < 0.001$.

‡ Statistically significant at $P < 0.01$.

Table 5. Effect of SK&F 86002, phenidone, ibuprofen and indomethacin on arachidonic-acid-induced rat paw edema*

Treatment	Dose (mg/kg, p.o.)	Change in paw volume (ml) (% inhibition)
SK&F 86002	120	52 ± 14†
	40	51 ± 13†
	13	22 ± 10†
Phenidone	120	34 ± 7†
	40	28 ± 12†
	13	3 ± 14
Ibuprofen	120	10 ± 13
Indomethacin	10	4 ± 10

* Animals were treated with the drugs 2 hr or, in the case of phenidone, 0.5 hr before subplantar injection of arachidonic acid. The results represent mean values ± SD derived from measurements on eight rats in the drug-treated groups and twelve rats in the vehicle-treated group 2 hr post arachidonic acid injection.

† Statistically significant at $P < 0.001$.

ibuprofen, which inhibit cyclooxygenase but not lipoxygenase activity, are far less effective in preventing cell infiltration into inflammatory lesions than agents that inhibit both enzymes [8, 19–22]. Similarly, the well-documented anti-inflammatory properties of the corticosteroids result from the inhibitory effects of these agents on the release of arachidonic acid by phospholipase A_2 [7], resulting in a decreased production of eicosanoids. Therefore, drugs that affect one or both of the pathways of arachidonic acid metabolism have been of great value in elucidating the respective importance of lipoxygenase and cyclooxygenase products in different inflammatory conditions and in developing new approaches for the rational design of improved anti-inflammatory agents.

The present study demonstrates that SK&F 86002 inhibits both pathways, i.e. cyclooxygenase and lipoxygenase. This profile has not been associated previously with imidazothiazoles nor has it been described for the anti-inflammatory, diaryl imidazoles (e.g. flumizole). Although SK&F 86002 clearly inhibited cyclooxygenase activity at the enzyme and whole cell level, the inhibitory potency of SK&F 86002 on cyclooxygenase activity varied according to the assay system used. When a high concentration of exogenously added arachidonate substrate was utilized in the isolated PGH_2 synthase preparation or in intact RBL-1 cells, SK&F 86002 was about two orders of magnitude less potent at inhibiting prostanoid production as compared to conditions where endogenous arachidonate was utilized such as in the human monocyte model. This difference in sensitivity may be attributed to the high substrate concentrations which compete with the drug–enzyme interaction. Additionally, drug-concentrating effects in the same cellular compartment as the PGH synthase may have resulted in greater inhibitory potency in the monocyte model. Preliminary studies suggest that SK&F 86002 is a substrate competitive inhibitor (data not shown). However, the unusual sensitivity of SK&F 86002 to substrate concentration may suggest multiple mechanisms of inhibition.

The ability of SK&F 86002 to inhibit 5-lipoxygenase/LTA_4 synthetase activity was demonstrated using the high speed supernatant fraction of RBL-1 extract preparations that demonstrated 5-lipoxygenase and LTA_4 synthetase activity. There was essentially equivalent inhibition of 5-lipoxygenase and LTA_4 synthase as reflected by 5-HETE and diHETE production. SK&F 86002 exhibited similar potencies for inhibition of lipoxygenase product formation by human neutrophils, monocytes and RBL-1 cells.

Some reports have shown a marked increase, as a consequence of selective cyclooxygenase inhibition, in the formation of lipoxygenase products [23–25]. The enhancement of lipoxygenase activity by cyclooxygenase inhibitors has been attributed to the shunting of arachidonic acid from cyclooxygenase to the lipoxygenase pathway [26–28] or to the removal of inhibition on the lipoxygenase pathway by prostaglandins [29]. The use of both RBL-1 crude extracts and intact cells allowed the evaluation of concomitant inhibition of 5-lipoxygenase and cyclooxygenase pathways. In these systems, SK&F 86002 proved to have inhibitory activity on both pathways. Thus, SK&F 86002 did not enhance the activity of one pathway as a result of inhibiting the other, as might be the case with a selective inhibitor.

SK&F 86002 actions *in vivo* as a dual inhibitor of eicosanoid metabolism are indicated by its anti-inflammatory profile (in particular, the ability to inhibit inflammatory cell infiltration and edematous responses that are not inhibited by selective cyclooxygenase inhibitors). Carrageenan-induced neutrophil infiltration in mice was clearly sensitive to SK&F 86002 and phenidone but not to cyclooxygenase inhibitors. Similar results were obtained using the rat "air pouch" model, in which injection of arachidonic acid into the air pouch induced neutrophil and mononuclear leukocyte infiltration. Similarly, edematous responses to arachidonic acid in mice and rats were also inhibited by SK&F 86002 and phenidone but not by cyclooxygenase inhibitors. Moreover, as seen with ibuprofen, stimulation of the inflammatory response may be induced by high doses of selective cyclooxygenase inhibitors. This may represent an *in vivo* example of shunting of arachidonate to the lipoxygenase pathway.

Phenidone proved to be less potent than SK&F 86002 *in vivo* as an anti-inflammatory agent despite the consistent observation of greater potency as a 5-lipoxygenase inhibitor *in vitro*. Many factors may account for such differences including drug pharmacokinetics and bioavailability. Indeed, duration of action studies indicated that the anti-inflammatory effect of phenidone wanes rapidly, whereas that of SK&F 86002 could be demonstrated over an 18-hr time span [30]. In addition, pharmacologic studies implicated the involvement of mast cell mediators in inflammatory responses in mice and rats [31]. Thus, inhibition of mast cell mediator release may also contribute to the overall anti-inflammatory activity of corticosteroids and dual inhibitors of arachidonate metabolism. Evaluation of the potency of phenidone and SK&F 86002 in inhibiting mast cell release *in*

vitro and *in vivo* may provide further insight into the cellular and molecular mechanisms of anti-inflammatory activity of this class of compounds which is not shared by selective cyclooxygenase inhibitors.

Overall, SK&F 86002 represents a potentially valuable addition to the limited panel of anti-inflammatory agents that can inhibit both pathways of arachidonic acid metabolism. Studies in progress to evaluate the effects of SK&F 86002 on modulating immune abnormalities associated with specific disease models and therapeutic index will be influential in determining its status as a candidate for clinical development.

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REFERENCES

1. I. Lantos, P. E. Bender, K. A. Razgaitis, B. M. Sutton, M. J. DiMartino, D. E. Griswold and D. T. Walz, *J. med. Chem.* **27**, 72 (1984).
2. R. A. Scherrer and M. W. Whitehouse, *Mednl Chem. Ser. Monogr.* **13**, II (1974).
3. P. Piper, *Br. med. Bull.* **39**, 255 (1983).
4. P. Davies, P. J. Bailey, M. M. Goldenberg and A. W. Ford-Hutchinson, *A. Rev. Immun.* **2**, 335 (1984).
5. M. J. H. Smith, A. W. Ford-Hutchinson and M. A. Bray, *J. Pharm. Pharmac.* **32**, 517 (1980).
6. P. M. Simmons, J. A. Salmon and S. Moncada, *Biochem. Pharmac.* **32**, 1353 (1983).
7. R. J. Flower and G. J. Blackwell, *Nature, Lond.* **278**, 456 (1979).
8. G. A. Higgs and J. R. Vane, *Br. med. Bull.* **39**, 265 (1983).
9. B. A. Jakschik and L. H. Lee, *Nature, Lond.* **287**, 51 (1980).
10. M. Hamberg and B. Samuelsson, *J. biol. Chem.* **241**, 257 (1966).
11. F. Colatta, G. Peri, A. Villa and A. Mantovani, *J. Immun.* **132**, 936 (1984).
12. E. F. Webb and D. E. Griswold, *J. Pharmac. Meth.* **12**, 149 (1984).
13. A. D. Sedgwick, Y. M. Sin, J. C. W. Edwards and D. A. Willoughby, *J. Path.* **141**, 483 (1983).
14. J. M. Young, D. A. Spires, C. J. Bedford, B. Wagner, S. J. Ballaron and L. M. DeYoung, *J. invest. Derm.* **82**, 367 (1984).
15. M. J. DiMartino, D. E. Griswold, B. A. Berkowitz, G. Poste and N. Hanna, *Agents Action* **20**, 113 (1987).
16. B. R. Allen and S. M. Littlewood, *Br. J. Derm.* **109**, 126 (1983).
17. E. M. Davidson, S. A. Rae and M. J. H. Smith, *Ann. rheum. Dis.* **42**, 677 (1983).
18. F. A. Kuehl, Jr. and R. W. Egan, *Science* **210**, 978 (1980).
19. J. R. Walker and W. Dawson, *J. Pharm. Pharmac.* **31**, 778 (1979).
20. E. Bramm, L. Binderup and E. Arrigoni-Martelli, *Agents Actions* **11**, 402 (1981).
21. J. A. Salmon, P. M. Simmons and S. Moncada, *J. Pharm. Pharmac.* **35**, 808 (1983).
22. C. Bertez, M. Miquel, C. Coquelet, D. Sincholle and C. Bonne, *Biochem. Pharmac.* **33**, 1757 (1984).
23. J. F. Burka and N. A. M. Paterson, *Prostaglandins* **19**, 499 (1980).
24. G. K. Adams III and L. M. Lichenstein, *Am. Rev. resp. Dis.* **131**, 8 (1985).
25. J. Y. Vanderhoek and J. M. Bailey, *J. biol. Chem.* **259**, 6752 (1984).
26. D. R. Robinson, M. Skoskiewicz, K. J. Block, G. Castorena, E. Hayes, E. Lowenstein, C. Melvin, F. Michelassi and W. M. Zapl, *J. exp. Med.* **163**, 1509 (1986).
27. M. Hamberg, J. Svensson and B. Samuelsson, *Proc. natn. Acad. Sci. U.S.A.* **71**, 3824 (1974).
28. J. J. Adcock, L. G. Garland, S. Moncada and J. A. Salmon, *Prostaglandins* **16**, 179 (1978).
29. E. A. Ham, D. D. Soderman, M. E. Zanetti, H. W. Dougherty, E. McCauley and F. A. Kuehl, Jr., *Proc. natn. Acad. Sci. U.S.A.* **80**, 4349 (1983).
30. D. E. Griswold, E. Webb, L. Schwartz and N. Hanna, *Inflammation* **11**, 189 (1987).
31. G. P. Harper, *Agents Actions* **20**, 69 (1987).