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PHARMACOLOGY OF THE PYRROLOIMIDAZOLE, SK&F 105809—II

ANTIINFLAMMATORY ACTIVITY AND INHIBITION OF MEDIATOR PRODUCTION *IN VIVO*

DON E. GRISWOLD,* PAUL J. MARSHALL,† JOHN C. LEE,‡ EDWARD F. WEBB, LEONARD M. HILLEGASS, JILL WARTELL, JOHN NEWTON JR.§ and NABIL HANNA‖

Departments of Respiratory/Inflammation Pharmacology, ‡Cell Sciences and §Drug Metabolism, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406-0939, U.S.A.

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Abstract—SK&F 105809 {2-(4-methylsulfinylphenyl)-3-(4-pyridyl)-6,7-dihydro-[5*H*]-pyrrolo[1,2,*a*] imidazole} demonstrated unique antiinflammatory activities in murine models that are resistant to selective cyclooxygenase (CO) inhibitors. Both edema and inflammatory cell infiltration induced by the topical application of arachidonic acid to the mouse ear were decreased by SK&F 105809 (ED_{50} values of 44 mg/kg, p.o.). Polymorphonuclear leukocyte (PMN) infiltration following the intraperitoneal injection of either monosodium urate crystal or carrageenan was inhibited with ED_{50} values of 64 and 72 mg/kg, p.o., respectively. These inflammatory responses were unaffected by the selective cyclooxygenase inhibitor naproxen. SK&F 105809 also inhibited leukotriene B_4 (LTB_4) and prostaglandin E_2 production *in vivo* in arachidonic acid-induced inflammatory exudates (ED_{50} values of 41 and 15 mg/kg, p.o., respectively). The inhibition of LTB_4 production preceded the inhibition of PMN infiltration. The impact of inhibition of both 5-lipoxygenase (5-LO) and CO was seen with platelet-activating factor-induced vascular permeability which was inhibited markedly by SK&F 105809. However, the 5-LO inhibitor, phenidone, only strongly inhibited when coadministered with the selective CO inhibitor, indomethacin. In spite of a short half-life (14–18 min) for both SK&F 105809 and the active metabolite SK&F 105561 {2-(4-methylthiophenyl)-3-(4-pyridyl)-6,7-dihydro-[5*H*]-pyrrolo[1,2-*a*] imidazole}, the pharmacological activity lasted at least 1.5 hr. The biochemical evidence of inhibition of interleukin-1 (IL-1) production and 5-LO and CO activity, *in vitro*, by the metabolite (SK&F 105561) seen in the companion paper (Marshall PJ, Griswold DE, Breton J, Webb EF, Hillegass LM, Sarau HM, Newton J Jr, Lee JC, Bender PE and Hanna N, Pharmacology of the pyrroloimidazole, SK&F 105809—I. Inhibition of inflammatory cytokine production and of 5-lipoxygenase- and cyclooxygenase-mediated metabolism of arachidonic acid. *Biochem Pharmacol* **42**: 813–824, 1991) and inhibition of the fluid and cellular phases of the inflammatory response, *in vivo*, by SK&F 105809 suggest that this compound possesses a unique profile of activity.

Recent studies have elucidated the pathophysiological role of arachidonic acid metabolites [1]. The chemotactic and algesic activities of leukotriene B_4 (LTB_4)¶ [2, 3], together with the observation that LTC_4 and LTD_4 cause increased vascular permeability [4–6], have led to considering the leukotrienes as targets for pharmacological intervention of the fluid and cellular phases of inflammatory diseases.

Likewise inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) have been shown to initiate phlogistic responses [7] and have been implicated in rheumatoid arthritis [8]. In addition, clinical evidence of the participation of 5-lipoxygenase (5-LO) products in inflammatory diseases has also been reported. LTB_4 was detected in rheumatoid and gouty arthritic joint fluid [9, 10], inflamed gastrointestinal mucosa [11], and psoriatic skin [12].

In contrast to selective cyclooxygenase (CO) inhibitors which do not reliably inhibit cell influx into inflammatory sites [13], corticosteroids and the prototypic cytokine and eicosanoid inhibitor, SK&F 86002, have been demonstrated to be effective in inhibiting inflammatory cell infiltration and eicosanoid production [14–16]. These results suggest that optimal antiinflammatory activity may require

* Correspondence: Dr. Don E. Griswold, Department of Respiratory/Inflammation Pharmacology, SmithKline Beecham Pharmaceuticals, P.O. Box 1539, King of Prussia, PA 19406-0939.

† Current address: Miles Research Center, 400 Morgan Lane, West Haven, CT 06516.

‖ Current address: IDEC Pharmaceuticals Corp., 11099 North Torrey Pines Rd., Ste. 160, La Jolla, CA 92037.

¶ Abbreviations: AAEE, arachidonic acid-induced ear edema; AUC, area under the plasma concentration curve; C_{max}, maximal plasma concentration; CO, cyclooxygenase; CSAID, cytokine suppressive antiinflammatory drug; DPBS/wo, Dulbecco phosphate-buffered saline without calcium or magnesium; ED_{50}, effective dose causing a 50% reduction of the control response; IL-1, interleukin-1; LTB_4, leukotriene B_4; LTC_4; leukotriene C_4; LTD_4, leukotriene D_4; 5-LO, 5-lipoxygenase; MPO, myeloperoxidase; MSU, monosodium urate; NSAID, non-steroidal antiinflammatory drug; PAF, platelet-activating factor; PGB_2, prostaglandin B_2; PGE_2, prostaglandin E_2; PMN, polymorphonuclear leukocyte; p.o., *per os*, by mouth; RIA, radioimmunoassay; $T_{1/2max}$, time to decay 50% of maximum response; $T_{1/2}$, apparent terminal half-life; T_{max}, time of C_{max}; and TNF, tumor necrosis factor.

inhibition of cytokine production as well as both fatty acid oxygenases and cannot be achieved by selective CO or 5-LO inhibitors.

In the present studies, SK&F 105809 {2-(4-methylsulfinylphenyl)-3-(4-pyridyl)-6,7-dihydro-[5*H*]-pyrrolo-[1,2-*a*] imidazole}, which is a prodrug to the active metabolite, SK&F 105561 {2-(4-methylthiophenyl)-3-(4-pyridyl)-6,7-dihydro-[5*H*]-pyrrolo[1,2-*a*] imidazole}, was evaluated *in vivo* and found to inhibit edema and polymorphonuclear leukocyte (PMN) infiltration in response to several proinflammatory stimuli. In addition, conversion of SK&F 105809 to its active metabolite was demonstrated. A preliminary report of some of these results has been published [17].

MATERIALS AND METHODS

Animals. Male Balb-c mice were obtained from the Charles River Breeding Laboratories (Kingston, NY). Within a single experiment, mice (20–28 g) were age-matched. ED_{50} Experiments involved the use of at least three dose levels of compound with a minimum of five animals/group and a control group of eight to ten mice.

Compounds and reagents. Phenidone, indomethacin and naproxen were each used as the free base. SK&F 105809 was synthesized by K. Erhard and all other pharmacological reagents were obtained from the Sigma Chemical Co. (St. Louis, MO). The compounds were homogenized in 0.5% tragacanth or dissolved in acid saline. Compounds were administered by gavage at a volume of 10 mL/kg. Concentrations of SK&F 105809 ranged from 1.25 to 10 mg/mL to deliver doses of 12.5 to 100 mg/kg.

Arachidonic acid-induced 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 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 or acid saline 15–30 min prior to the topical application of arachidonic acid.

PMN infiltration at 1 hr post-administration of arachidonic acid was determined using the method of Bradley *et al.* [18] modified as follows. Ice-cold, minced ear tissues were homogenized (10%, w/v) in 0.5 M phosphate buffer (pH 6.0) containing 5 mg/mL hexadecyltrimethylammonium bromide (HTAB), using a Tissumizer (Tekmar). The tissue homogenates were taken through three cycles of freeze/thaw followed by brief sonication (10 sec). The samples were then centrifuged in a Microfuge (Beckman Instruments, Inc.) at maximum speed for 15 min (15,000 *g*). The appearance of a colored product from the myeloperoxidase (MPO)-dependent reaction of *o*-dianisidine (0.167 mg/mL) and hydrogen peroxide (0.0005%) was measured spectrophotometrically at 460 nm. Supernatant MPO activity was quantified kinetically (change in absorbance over 3 min sampled at 15-sec intervals) using a Beckman DU-7 spectrophotometer with Kinetics Analysis. One unit of MPO activity is defined as that degrading 1 μmol of peroxide/min at 25°.

Monosodium urate (MSU) and carrageenan-induced peritonitis. Mice were pretreated with either the test compound or vehicle (administered orally) 20 min before the intraperitoneal injection of a suspension of 2 mg MSU crystals in saline prepared by the method of McCarty and Faires [19] or 0.2 mL of 1% carrageenan (Viscarin, Marine Colloids, Springfield, NJ). Two hours later, the mice were killed by carbon dioxide (CO_2) asphyxiation and injected with 3 mL of cold Dulbecco phosphate-buffered saline without calcium or magnesium (DPBS/wo). Following gentle massage, the peritoneum was incised, and the peritoneal fluid was removed and placed on ice. Cell density of the washes was determined by a Coulter Counter (model ZBI, Coulter Electronics, Hialeah, FL). PMN differential cell counts were performed microscopically from slides prepared by a cytospin (Cytospin 2, Shandon Southern Products, Ltd., England) and Giemsa-stained (Accra Lab Inc., Bridgeport, NJ). A 1.5-mL aliquot of each washout was centrifuged at 12,500 *g* for 10 min in the cold, the supernatant was aspirated off, and the pellet was extracted for MPO analysis by the method of Bradley *et al.* [18]. Direct cell counts and MPO were equivalent methods.

Measurement of eicosanoid production in vivo. Mice were treated with 2.0 mg/ear of arachidonic acid. After 20 min (for the time courses, 0–60 min was used), the mice were killed by carbon dioxide asphyxiation. The ears were removed, immediately frozen in liquid nitrogen, and then stored in liquid nitrogen. The frozen ears were pulverized with a hammer and immediately homogenized with a Tissumizer tissue grinder in 1 mL of ice-cold distilled water containing prostaglandin B_2 (PGB_2) (1 nmol). The homogenate was diluted with 1 mL of chilled acetonitrile and centrifuged at 7000 *g*, 5° for 10 min. The supernatant was collected and diluted with 3 mL of chilled aqueous 1% formic acid and 1% triethylamine. This mixture was loaded onto a preconditioned C-18 Baker Extraction cartridge (J. T. Baker Chemical Co., Phillipsburg, NJ). The cartridge was washed in the following order: 3 mL of aqueous 1% formic acid with 1% triethylamine and 3 mL of petroleum ether. The eicosanoids were eluted with 3 mL methyl formate. The solvent was removed under vacuum. The samples were dissolved in 200 μL of 30% acetonitrile buffered with 50 mM ammonium acetate and chromatographed using a NOVA-PAK C-18 RCM column. The samples were chromatographed using a convex gradient from 19% to 35% acetonitrile buffered with ammonium acetate over 12 min. Between injections the column was flushed with 90% acetonitrile and then reequilibrated with 10% acetonitrile. Elution times for PGE_2 (7 min) and LTB_4 (12 min) standards were used to collect sample fractions. The fractions (2.5 mL) were taken to dryness under vacuum and reconstituted to 200 μL. The samples were then assayed for PGE_2 and LTB_4 with the appropriate radioimmunoassay (RIA) kit.

Platelet-activating factor (PAF)-induced dye

extravasation. Mice were injected with 0.2 mL of 0.2% Evan's blue dye in saline (J. T. Baker Chemical Co.) by tail vein just prior to an intraperitoneal inoculation of 0.25 mg PAF in saline. Test compound was administered 15–30 min prior to PAF injection. The mice were killed (CO_2) 30 min later, and the peritoneal contents were washed out with 3 mL of cold DPBS/wo. The washouts were centrifuged at 12,500 *g* for 15 min in the cold and the supernatants were filtered through 0.2 μm membranes prior to spectrophotometric analysis of dye concentration at 610 nm in a Perkin–Elmer spectrophotometer (model 552A).

Quantitation of SK&F 105809 and metabolites in plasma. Plasma concentrations of SK&F 105809 and its sulfide sulfone metabolites, SK&F 105561 and SK&F 105942, were quantitated in arachidonic acid-treated mice. At various time intervals post-drug administration plasma was assayed by HPLC after solid phase extraction using Baker 10 SPE (J. T. Baker Chemical Co.) 1-mL C_{18} colums pre-conditioned with methanol and water. For plasma concentrations at times less than 1 hr post SK&F 105809, separate, non-arachidonic acid treated animals were used. Samples (100 μL), plus internal standard (SK&F 104493), were added to the columns, which were then washed with 1 column volume of ammonium acetate (50 mM). SK&F 105809 and its metabolites were eluted from the column with methanol (2 × 500 μL). Samples were dried under nitrogen and resuspended in HPLC mobile phase (100 μL), and aliquots (50 μL) of the reconstituted samples were quantitated on an HPLC system consisting of a model 6000A solvent delivery system, a model 590 solvent delivery system, a model 710B autoinjector, a Kratos Spectroflow 783 detector set at 270 nm, and a model RCM 8X10 radial compression module that contained a 5 μL NOVA-PAK C_{18} (5 × 100 mm) cartridge (Waters Associates, Milford, MA). SK&F 105809 and its metabolites were separated by gradient elution with 10 mM ammonium acetate (solvent A) and 75% acetonitrile in 10 mM ammonium acetate (solvent B). Following injection, the percentage of solvent B was increased in a linear fashion over 10 min from 25 to 65%, and then held at 65% for an additional 1 min before returning to initial conditions. Flow rate was maintained at 2 mL/min throughout the analysis. Plasma concentrations of SK&F 105809 and metabolites were determined from peak area ratios (compared with internal standard) that were compared with standard curves that were made up in plasma and extracted.

For both SK&F 105809 and SK&F 105942, the assay had a limit of quantitation of 0.2 μg/mL and was linear up to 40 μg/mL. For SK&F 105561, the assay had a limit of quantitation of 0.05 μg/mL and was linear to 10 μg/mL.

Pharmacokinetics. Mean plasma concentration versus time profiles for SK&F 105809 and SK&F 105561 were analyzed using PROTOCOL (for non-compartmental analysis, based on the VAX cluster) pharmacokinetic parameter estimation software. The area under the plasma concentration time curve (AUC) was determined using the linear-log linear trapezoidal rule. When appropriate, a plasma concentration of zero was used at zero time as a data point in the calculation of AUCs. The half-lives of SK&F 105809 and SK&F 105561 were obtained from the slope of the log transformed plasma concentration time profile by the method of curve peeling. The AUC from the last time point to infinity was extrapolated by dividing the last data point by the terminal rate constant determined from the fitted data.

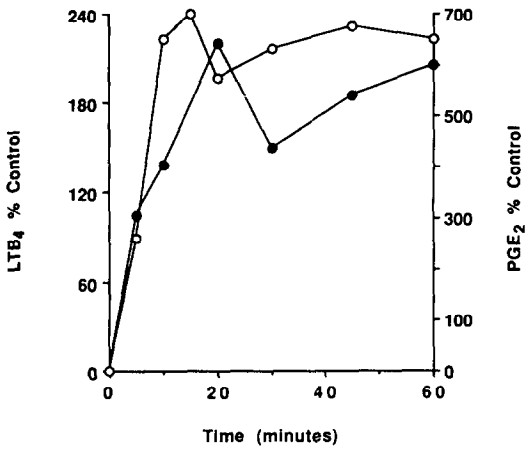

Fig. 1. Time course of LTB_4 and PGE_2 production. The production of LTB_4 (●) and PGE_2 (○) was followed in mouse ears to which arachidonic acid (2 mg/ear) had been applied. Ears were taken at the times shown and eicosanoids extracted and assayed by HPLC fractionation followed by RIA analysis. The baseline control value (zero time) for LTB_4 was 1210 pg and for PGE_2 58 ng. Data shown are complied from two experiments; each point represents an N of three pools of three animals each. Where data have been combined, the N = 6.

Statistics. Statistical analysis was done using Student's *t*-test. A $P < 0.05$ was considered significant. The ED_{50} is the dose that caused a 50% inhibition of the vehicle-treated control response and was calculated by regression analysis of the dose–response data using the P57 Biostatistics program on VAX. The dose–response was generated using at least three dose levels of test compound.

RESULTS

Inhibition of eicosanoid metabolism by SK&F 105809. The following experiments were carried out to demonstrate that eicosanoid production *in vivo* was inhibited by SK&F 105809. The topical application of arachidonic acid was used to generate eicosanoids in the treated ears. LTB_4 and PGE_2 were found 5 min after application of arachidonic acid and reached a maximum at 15–20 min (Fig. 1). The peak production of LTB_4 occurred prior to significant PMN infiltration (data not shown). As seen in Table 1, SK&F 105809 inhibited LTB_4 (ED_{50} 41 mg/kg, p.o.) and PGE_2 (ED_{50} 15 mg/kg, p.o.) production. Naproxen was a more potent inhibitor of PGE_2 production (ED_{50} 0.5 mg/kg, p.o.), and phenidone selectively inhibited LTB_4 production (ED_{50} 27 mg/kg, p.o.).

Antiinflammatory activity of SK&F 105809. The

Table 1. Inhibition of eicosanoid production induced by arachidonic acid

Compound	ED_{50}, mg/kg, p.o. (95% confidence limits)	
	LTB_4	PGE_2
SK&F 105809	41 (22–176)	15 (4–25)
Phenidone	27 (6–45)	>100
Naproxen	*	0.5

* Non-dose-related inhibition observed.

Table 2. Effects of SK&F 105809, phenidone and naproxen on edema and inflammatory cell infiltration induced by arachidonic acid

Compound	ED_{50}, mg/kg, p.o. (95% confidence limits)	
	Edema	MPO
SK&F 105809	44 (26–64)	44 (28–64)
Phenidone	62 (48–86)	20 (14–26)
Naproxen	NA*	NA

* NA, no significant activity at doses up to 100 mg/kg, p.o.

Table 3. Effects of SK&F 105809, phenidone and naproxen on carrageenan- and monosodium urate crystal-induced peritonitis

Compound	ED_{50}, mg/kg, p.o. (95% confidence limits)	
	Carrageenan	MSU
SK&F 105809	72 (54–106)	64 (40–159)
Phenidone	71 (23–124)	129 (98–182)
Naproxen	NA*	NA

* NA, no significant activity at doses of 100 mg/kg, p.o.

antiinflammatory activity of SK&F 105809 was evaluated in models which allowed quantification of the edematous and cellular phases of the inflammatory response. As seen in Table 2, SK&F 105809 inhibited both edema (ED_{50} 44 mg/kg, p.o.) and inflammatory cell infiltration as measured by MPO activity (ED_{50} 44 mg/kg, p.o.). As expected, similar activity was observed with the 5-LO inhibitor, phenidone. In contrast, the selective CO inhibitor naproxen was without effect on either response.

The inhibition by SK&F 105809 of cell infiltration that was elicited by either carrageenan or MSU crystals was examined. PMN infiltration induced by both stimuli was inhibited in a dose-related manner (ED_{50} 72 and 64 mg/kg, p.o., respectively). Inhibition was also demonstrable with the 5-LO inhibitor phenidone but was not a property of the selective CO inhibitor naproxen (Table 3).

Corroboration of the effect of SK&F 105809 on the increased vascular permeability was accomplished by injecting PAF intraperitoneally in SK&F 105809-treated mice. As seen in Table 4, SK&F 105809 was an effective inhibitor of PAF-induced dye extravasation. Both the 5-LO inhibitor phenidone and the potent, selective CO inhibitor indomethacin displayed weak inhibitory activity in this model. The combination of the two compounds, however, improved the inhibition.

Duration of antiinflammatory activity and pharmacokinetics. Arachidonic acid-induced inflammation is a rapid and convenient method to evaluate the duration of action of active compounds. Following oral administration, plasma concentrations of SK&F 105809 were maximal at the first time point investigated (7.5 min) achieving a mean value of 41 μg/mL (Fig. 2, Table 5). Plasma concentrations of SK&F 105809 then declined in a monoexponential fashion with a half-life of approximately 20 min. Maximal plasma concentrations of the active metabolite, SK&F 105561, were achieved slightly later (15 min) but remained relatively constant at 4.5 to 6.0 μg/mL for a period up to 1 hr after SK&F 105809 administration. From this point, SK&F 105561 disappeared from plasma in a monoexponential fashion with a half-life of approximately 15 min.

Table 4. Effects of SK&F 105809, phenidone and indomethacin upon PAF-induced peritoneal dye extravasation in the mouse

Treatment	N	$A_{610\ nm}$	Percent inhibition
Vehicle, p.o.	11	0.269 ± 0.012	
SK&F 105809, 50 mg/kg, p.o.	6	0.117 ± 0.017*	57
Phenidone, 100 mg/kg, p.o.	4	0.213 ± 0.015†	21
Indomethacin, 3 mg/kg, p.o.	5	0.190 ± 0.010*	29
Phenidone, 100 mg/kg, + indomethacin, 3 mg/kg, p.o.	4	0.133 ± 0.020*	50

The above data were pooled from two studies in which the control means were 0.259 and 0.281. Absorbance values are means ± SEM.

* Significantly different from the vehicle control at $P < 0.001$.

† Significantly different from the vehicle control at $P < 0.02$.

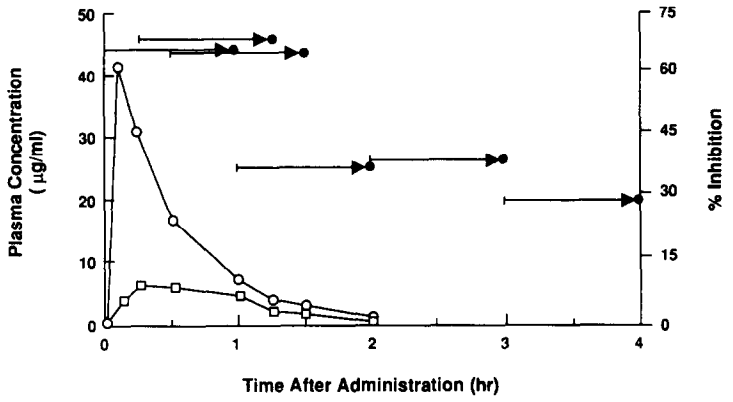

Fig. 2. Pharmacodynamics of SK&F 105809. The plasma concentrations of SK&F 105561 (□) and SK&F 105809 (○) were determined by HPLC analysis at various times post-administration of drug. Also shown by the arrow and (●) is the inhibition of arachidonic acid-inducer ear swelling using various pretreatment times of SK&F 105809 at 50 mg/kg, p.o. The arrow indicates the hour time period between application of arachidonic acid and determination of ear swelling. Each point is the mean of an N of three or more. Mean and SEM ear swelling for the control vehicle-treated animals was 21.5 ± 2.3 ($\times 10^{-3}$ cm) at 1 hr post arachidonic acid application. All percent inhibition shown is statistically significantly different from the vehicle control at a $P < 0.001$. The results are from a single experiment.

Table 5. Pharmacokinetic parameters for SK&F 105809 and 105561 obtained from mean plasma concentrations following oral administration of SK&F 105809 (50 mg/kg)

Parameter*	SK&F 105809	SK&F 105561
T_{max}	7.5	15
C_{max}	41.31	6.13
AUC_{0-120}	20.9	6.5
$T_{1/2}$	18	14

* Abbreviations of pharmacokinetic parameters are: C_{max}, maximal plasma concentration (μg/mL) of drug observed: T_{max}, time (min) after drug administration that C_{max} occurred. AUC_{0-120} (μg-hr/mL), area under the plasma concentration time curve up to 2 hr after dosing; $T_{1/2}$ (min), apparent terminal half-life.

Pharmacologic activity of SK&F 105809 was maximal (66% inhibition) for pretreatment periods up to 30 min. During the 90-min period of the experiment, the mean maximal plasma concentrations of SK&F 105809 ranged from 41 to 16.6 μg/mL and the mean minimum plasma concentrations of SK&F 105809 ranged from 6.9 to 2.5 μg/mL (Fig. 2). In contrast, SK&F 105561 plasma concentrations remained relatively constant during the time of maximal activity; mean maximal plasma concentrations of SK&F 105561 during this period ranged from 6.1 to 5.8 μg/mL and mean minimum plasma concentrations of SK&F 105561 ranged from 4.5 to 2.9 μg/mL. Kinetic studies indicate that plasma concentrations of SK&F 105809 and SK&F 105561 in the range of 2 to 20 and 3 to 6 μg/mL, respectively,

provide nearly full antiinflammatory activity in arachidonic acid-induced ear edema in the mouse. However, a significant pharmacologic effect of SK&F 105809 still was observed at times (> 2 hr after drug administration) where plasma concentrations of SK&F 105809 and 105561 were below the limits of detection of the assay.

DISCUSSION AND CONCLUSION

SK&F 105809 was demonstrated to inhibit the fluid and cellular phases of the inflammatory response. This action is not attributable to inhibition of cyclooxygenase activity since the selective cyclooxygenase inhibitor naproxen was devoid of antiinflammatory activity in the arachidonic acid-induced inflammation and in MSU- and carrageenan-induced peritonitis models even though the doses used were clearly inhibitory to PGE_2 production. Furthermore, direct biochemical evidence in support of the mode of action was provided by demonstrating SK&F 105809-mediated inhibition of LTB_4 production in the inflammatory site. An excellent agreement between the ED_{50} values for inhibition of inflammatory cell infiltration and LTB_4 production in the arachidonic acid inflammation model was observed. This coupled with the observation that LTB_4 production precedes significant PMN infiltration suggests that LTB_4 functions as the chemotactic mediator for PMN infiltration in this model.

Evidence that the dual inhibition of 5-LO and CO seen with SK&F 105809 results in activity not seen with selective inhibitors was also demonstrated. In contrast to SK&F 105809, the response to the intraperitoneal injection of PAF was relatively resistant to the action of selective 5-LO and CO inhibitors and a combination of both was required to exert inhibition.

The above data support the suggestion that the pharmacology of SK&F 105809 is related in part, to the ability of the compound to inhibit eicosanoid production; furthermore, metabolism studies documented conversion to the active sulfide metabolite, whose activity on fatty acid oxygenases is detailed in the companion paper [20].

The pharmacokinetic studies in the mouse suggest that SK&F 105809 was absorbed rapidly and reduced to the active principle, SK&F 105561, and that both are cleared rapidly; this may help to explain the increased doses necessary to inhibit PMN infiltration in models which are of longer duration than the edema responses. This evaluation also demonstrated that SK&F 105561 plasma concentrations of 2–6 μg/mL afforded nearly complete inhibition of arachidonic acid-induced inflammation and that significant antiinflammatory activity was demonstrable at concentrations below 2 μg/mL.

As described in the companion paper [20], the active principle of SK&F 105809, SK&F 105561, has been shown to inhibit IL-1 production by human monocytes with an IC_{50} of about 2 μM. In a similar fashion, TNF levels have been found to be inhibited *in vivo*.* Since IL-1 and TNF are capable of mounting an inflammatory response, it is likely that some of the antiinflammatory activity observed could be accounted for through that mechanism. However, the time frame for arachidonic acid-induced inflammation is perhaps too short for cytokine production to occur substantially. In the case of peritonitis, where the response builds over a 2-hr period, cytokine involvement is more likely. Phenidone, which does not inhibit IL-1 production [21], was much less effective than expected in the peritonitis models, while SK&F 105809 was approximately equally effective in inhibiting LTB_4 production and peritonitis. These results suggest that there may be a contribution of IL-1 inhibition in the antiinflammatory profile of SK&F 105809. That inhibition of inflammatory cytokine production is an important feature of SK&F 105809 pharmacology is supported by the observation that TNF serum levels were reduced markedly by oral administration of SK&F 105809 in LPS-treated animals.*

Taken together, these results suggest that SK&F 105809 represents a member of a new pharmacological class of agents which we have termed CSAIDs (cytokine suppressive antiinflammatory drugs). Thus, the overall profile of activity suggests that SK&F 105809 would be effective in the treatment of inflammatory diseases mediated by cytokines and eicosanoids, and in particular those diseases in which inflammatory cell infiltration plays a significant role.

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