the interaction of the terminal phenyl ring of the *N*-substituent can be ruled out by XXX and XXXI. The substitution of either a strong electron-withdrawing nitro group (XXX) or a strong electron-donating amino group (XXXI) resulted in only minor fluctuations in inhibitor strength. Additionally, the unaltered inhibitor potency after the addition of a polar amino group to XXIV indicates that only part of the terminal phenyl ring is involved in a hydrophobic interaction with the carrier molecule.

Overall, there appears to be no simple correlation between hydrophobicity and inhibitor strength in this series of compounds as has been reported for primary alcohols (26) and certain psychotropic drugs (27). The introduction of the proper bulky groups on the amphetamine nitrogen can be tolerated with little loss in inhibitor potency. Thus, the preparation of specific nonclassical inhibitors with substitution at the nitrogen seems possible.

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[x] To whom inquiries should be directed.

Flumizole, a New Nonsteroidal Anti-Inflammatory Agent

E. H. WISEMAN [x], H. M. McILHENNY, and J. W. BETTIS *

Abstract □ Flumizole is a potent anti-inflammatory agent in animal models with an inhibitory activity severalfold that of indomethacin in rat foot edema and prostaglandin synthetase tests. The drug was well absorbed from the GI tract when administered in the solution used in pharmacological assays. However, markedly poorer absorption of the solid form of this poorly water-soluble agent led to the development of a flumizole dispersion with polyethylene glycol 6000. The solid dispersion exhibited an increased dissolution rate in simulated gastric fluid and improved absorption properties in dogs relative to unformulated flumizole. Studies with a capsule formulation of the solid dispersion in human volunteers were indicative of good drug absorption. Plasma levels of flumizole were rapidly achieved and declined with a short half-life (2–7 hr) in rats, dogs, and humans.

Keyphrases □ Flumizole—anti-inflammatory activity, bioavailability, and formulation □ Anti-inflammatory agents—flumizole (nonsteroid) activity, bioavailability, and formulation □ Bioavailability—flumizole, a nonsteroidal anti-inflammatory agent □ Dissolution rates—formulations of flumizole

Flumizole[1] is one of a series of substituted diarylimidazoles with anti-inflammatory activity (1, 2). This compound is distinguished from the general class of acidic agents currently used in the management of inflammatory disease by its very weak acidic character (pKa 10.7). This report describes the potent anti-inflammatory activity of flumizole and biopharmaceutical developments undertaken to opti-

[1] 4,5-Bis(*p*-methoxyphenyl)-2-(trifluoromethyl)imidazole, synthesized by Dr. Joseph G. Lombardino, Department of Medicinal Chemistry, Pfizer Central Research, Pfizer, Inc., Groton, CT 06340

mize drug absorption during pharmacological and toxicological investigations.

EXPERIMENTAL

Charles River strain rats (male), Hartley strain guinea pigs (male), and beagle dogs (both sexes) were used. Adrenalectomy in the rat was performed through a retroperitoneal incision while the animals were anesthetized with ether. The adrenalectomized rats were maintained on 0.9% saline in place of drinking water and were used 5–7 days postoperatively. Oral studies were conducted with an aqueous solution of drug in polyethylene glycol 300 containing 5% polysorbate 80[2] (I; 0.2 ml in 5 ml water/animal).

Anti-Inflammatory Activity—Inhibitory activity toward the formation of edema in response to the subplantar injection of carrageenan in the hindpaw of the rat (mean weight 170 g) was assessed using the technique of Winter *et al.* (3). Carrageenan (0.05 ml of 1% suspension) was injected 1 hr after oral drug administration, and inhibition of edema formation was assessed after a further 3-hr period. Analogous studies were performed in adrenalectomized rats.

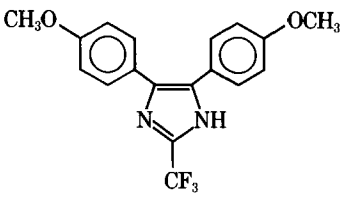
Inhibitory activity toward the formation of granuloma tissue around an implanted irritant was measured by a procedure developed in these laboratories (4). Under ether anesthesia, rats, 140–160 g, were shaved and a 8-cm length of sterile linen string was inserted subcutaneously at the dorsal midline. Drugs were administered orally 4, 24, and 48 hr after implantation. At 72 hr, the animals were sacrificed and weighed and the string together with the granulation tissue was removed and weighed.

Inhibitory activity toward UV irradiation-induced erythema was measured in the guinea pig according to the method of Winder *et al.* (5). Drugs were administered orally in divided doses, 1 hr prior and just subsequent to irradiation. Erythema inhibition was assessed 2 hr after irradiation. Groups of five animals were used, and erythema was scored as follows: no erythema, 0; full circle of erythema 1.0; and intermediate responses, 0.5. Erythema was assessed at three irradiation sites on each animal (maximum possible score = 3.0).

Prostaglandin synthetase was isolated from goat seminal vesicles according to the method of Wlodawer and Samuelsson (6). Labeled (1-^{14}C) and unlabeled arachidonic acid were purified as reported by Flower *et al.* (7). Reaction mixtures contained 1.2 mg/ml of microsomes, 0.36 m*M* arachidonic acid (specific radioactivity 3.45×10^5 cpm/μmole), 10 m*M* L-epinephrine bitartrate, 50 m*M* potassium phosphate buffer (pH 7.2), and 3.0 *M* methanol in a total volume of 1 ml. The range of drug concentrations used were $0.3–4.0 \times 10^{-5}$ *M* indomethacin, $0.2–4.0 \times 10^{-3}$ *M* phenylbutazone, and $0.1–2.0 \times 10^{-6}$ *M* flumizole. The reaction was initiated by the addition of enzyme and was conducted at 37° for 10 min.

Conditions were chosen so that no greater than 30% of the arachidonate was converted to products. The reaction was stopped by the addition of 7 ml of chloroform–methanol (1:1 v/v). Carrier prostaglandin $F_{2\alpha}$ (0.09 μmole), prostaglandin E_2 (0.09 μmole), and arachidonic acid (0.17 μmole) were added. The precipitate was removed by centrifugation, and the radioactive products were extracted and separated by TLC (6). Zones corresponding to prostaglandins $F_{2\alpha}$ and E_2 were visualized with iodine vapors, scraped from the plate, and eluted with 1 ml of methanol. The radioactivity measured was corrected for the amount present in a boiled microsome control. Radiochemical assays were carried out by liquid scintillation[3] with a solvent system of 60 g of naphthalene, 8 g of solute[4], and 100 ml of methanol made to a final volume of 1 liter with dioxane.

Drug Assay in Plasma—To 3–5 ml of plasma was added 2 ml of 1 *M* NaH_2PO_4, and the solution was mechanically extracted for 10 min with freshly distilled benzene (1 × 20 ml). The solvent extract was separated after centrifugation and filtered through a column of silica gel[5], 0.5 g, contained in a 14.6-cm capillary pipet. The filtrate and an additional 3 ml of benzene were rejected. Drug was eluted from the column with 3 ml of benzene–ether (1:1), which was evaporated to 0.2 ml and diluted with 0.5 ml of *n*-hexane. Drug was extracted by mechanical agitation with 1 ml of 1 *M* NaOH; following centrifugation, the aqueous solution was immediately acidified with 1 ml of 2 *M* HCl for spectrophotofluorometric determination[6].



flumizole

Flumizole displayed an excitation maximum at 290 nm with an emission maximum at 460 nm. Response was linear and could be quantitated over a concentration range of 5–1000 ng/ml in plasma. Dilution of the final assay solution permitted quantitative analysis up to 10 μg/ml plasma concentrations. Standardization was achieved by reference to a calibration curve constructed for each assay series by addition of known amounts of flumizole, in duplicate, to untreated animal plasma or normal human serum; these samples were processed in the same manner and at the same time as above. No fluorescent background interferences were present in the plasma of unmedicated rats, dogs, or humans.

Assay precision was determined by repeated analysis, over a 3-week period, of two control samples containing drug. Results showed a reproducibility of 9.5 ± 0.2 ng/ml (seven determinations) and 50.4 ± 4.0 ng/ml (eight determinations). The assay was specific for unchanged drug in plasma as confirmed by fluorescence spectrum, TLC [silica gel GF plates[7], hexane–ethyl acetate–acetic acid (14:6:1)], and GLC (described later) analyses of final assay samples or extracts thereof. Drug recovery from plasma averaged 71 ± 2% over the 10–50-ng/ml concentration range.

Drug Assay in Excreta—Fecal and urinary excretion of flumizole in humans was determined by a GLC procedure. Five milliliters of urine, or 5 ml of 1 *M* NaH_2PO_4 containing 100 mg of lyophilized feces, in a centrifuge tube with 5 ml of pH 4.5 sodium acetate–acetic acid buffer (0.2 *M*) was processed by benzene extraction and silica gel column purification in the manner described for the plasma assay. The urine residue obtained by evaporation of the column eluate to dryness with nitrogen was directly dissolved in 50 μl of methanolic triethylanilinium hydroxide (0.5 *M*) for analysis. The column eluate from the fecal sample was evaporated to 0.2 ml and diluted to 1 ml with *n*-hexane, and drug was extracted into 1 ml of 1 *M* NaOH.

After centrifugation, the aqueous phase was withdrawn, immediately acidified with 1 ml of 2 *M* HCl, and extracted with 10 ml of ethyl acetate. Following centrifugation, the solvent layer was withdrawn, evaporated to dryness, and dissolved in 0.5 *M* triethylanilinium hydroxide, as already described, for analysis. One-microliter aliquots (plus 1 μl of 0.5 *M* triethylanilinium hydroxide solution) were immediately introduced into the injection port (320°) of a gas chromatograph[8] for on-column alkylation to the corresponding N_1-ethylated derivative of flumizole. The derivative was separated on a 0.63 × 183-cm glass column (210°) containing 1% OV-1 on Gas Chrom Q (60–80 mesh) and detected by flame ionization (320°) with a typical retention time, using helium as carrier gas (60 ml/min), of 3.1 min.

Concentration was determined by peak height measurement with reference to a calibration curve constructed from a series of normal human urine or fecal samples fortified with several levels of authentic drug. A linear response over the concentration range of 2.0–10 μg of drug/ml of urine or fecal homogenate permitted quantitative analysis with no background interferences. Recovery of flumizole added to untreated urine or feces was similar to that obtained with plasma, and the derivatization step with triethylanilinium hydroxide was judged 100% complete by GC comparison with the authentic N_1-ethyl analog of flumizole.

[2] Tween 80.
[3] Beckman model LS-250.
[4] Omnifluor, New England Nuclear.
[5] E. Merck AG, 0.05–0.2 mm (70–325 mesh ASTM).
[6] Aminco-Bowman, slit arrangement No. 4.
[7] E. Merck AG 250 μm, Brinkmann Instruments, Inc.
[8] Tractor model MT-220.

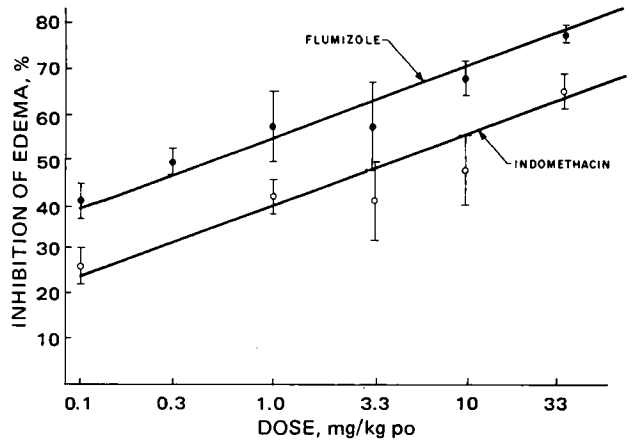


Figure 1—*Dose–response regression lines for the antiedema effects of flumizole and indomethacin in normal rats, six animals at each observation (mean ± SD).*

Estimation of urinary drug excretion in rats and dogs was accomplished by TLC. An aliquot of urine from treated and untreated animals was diluted with an equal volume of 1 *M* NaH_2PO_4, extracted with ethyl acetate (2 × equal volume), and reduced to dryness. The residue obtained directly (or following silica gel column purification as in the plasma assay) was developed on silica gel GF plates[7] with benzene–ethyl acetate–acetic acid (90:5:5) in the presence of authentic flumizole for reference purposes. The presence of drug was revealed by plate exposure to UV light and compared to reference zones of flumizole.

Animal fecal samples were analyzed by fluorometry in aqueous acid (beagles) or by TLC (rats) as already described. Ten percent of administered flumizole would have been detectable in rat feces. Gradual decomposition of flumizole was observed at pH 8 or above in aqueous buffers (the products will be discussed in a forthcoming publication). Drug recoveries averaged 70% (six tests, range of 40–95%) following the incubation of normal dog fecal homogenate with flumizole at concentrations encountered in medicated dog studies. Since a reliable correction factor for potential drug degradation in the gut was not obtainable, fecal assays for flumizole could only be interpreted in a semiquantitative manner and represent, therefore, minimum estimates of actual unabsorbed drug.

Drug Formulation—To prepare the solid dispersion of flumizole for animal bioavailability studies, polyethylene glycol 6000 (II) was melted (approximately 70°) and flumizole was added in small portions with constant stirring. The uniform melt was flash cooled by transferring the beaker to an ice bath. The solidified product was reduced to a fine powder and sieved. Only the powder that passed the 100-mesh but was retained on the 200-mesh sieve was used in the dissolution test.

Analysis was performed by weighing aliquots of the solid dispersions containing approximately 20 mg of flumizole into 100-ml volumetric flasks. After approximately 50 ml of methanol was added, the flasks were shaken mechanically for 30 min and then diluted to volume with methanol. The solutions were filtered[9] to remove undissolved II. A 10-ml aliquot of the filtrate was diluted to 100 ml with methanol. The absorbance of the resulting solution at 267 nm was determined spectrophotometrically[10].

A standard solution was prepared by shaking 20 mg of flumizole and 80 mg of II with methanol, filtering, diluting, and reading as described. A quantity of II equal to that contained in the unknown samples was treated in the same manner as the sample and standard solutions and used as the UV reference solvent. Analysis confirmed drug concentrations in the dispersion within ±5% of theory. Stability of drug in the II melt was established by silica gel GF[7] TLC, using a benzene–5% acetic acid solvent system and UV light irradiation for drug detection.

To determine dissolution rates of the solid dispersion in simulated gastric fluid without pepsin (pH 1.2), an excess of pure flumizole or the solid dispersion beyond its equilibrium solubility was added to 500 ml of simulated gastric fluid maintained at 37 ± 0.5°

[9] Reeve Angel No. 802 paper.
[10] Cary model 15.

Table I—Effects of Flumizole, Indomethacin, and Phenylbutazone on Prostaglandin Synthetase *In Vitro*

Compound	Effective $Dose_{50}$[a] Prostaglandin E_2, μM	Prostaglandin $F_{2\alpha}$, μM	Potency[b]
Flumizole	0.7	0.8	8
Indomethacin	5.6	6.1	1
Phenylbutazone	370	350	0.017

[a] Determined from linear regression analysis of data obtained at arachidonic acid concentrations of 3.6×10^{-4} *M*; a minimum of four concentrations with three replicates was used. [b] Potency relative to indomethacin.

in a 600-ml jacketed beaker. The contents of the dissolution bath were stirred with an overhead T-shaped stirrer rotating at 60 rpm and placed such that the Teflon paddle scraped the bottom of the beaker. Aliquots (10 ml) of the dissolution medium were withdrawn at various times and immediately filtered[11] and diluted to 25 ml with methanolic hydrochloric acid (7 ml of concentrated hydrochloric acid/liter of methanol).

The fluorescence of the resulting solutions was determined[6]. Standard solutions were prepared by dissolving known quantities of flumizole in methanolic hydrochloric acid, adding 10 ml of simulated gastric fluid, and diluting to 25 ml with methanolic hydrochloric acid. There was a linear response over the concentration range studied. The baseline fluorescence of 10 ml of fluid diluted to 25 ml with methanolic hydrochloric acid was subtracted from the readings of all standard and unknown solutions.

For bioavailability studies in animals, the solid dispersion was encapsulated[12]. For clinical investigations, the 100–200-mesh flumizole solid dispersion was mixed with lactose as a bulking agent, followed by the addition of 0.5% fumed silica[13] as a lubricant, to afford a free flowing powder for encapsulation[12]; a 25-mg drug capsule contained 125 mg of solid dispersion and 350 mg of lactose. Dissolution profiles were obtained in simulated gastric fluid according to NF XIII Dissolution Test Method I with minor modifications. Aliquots of the dissolution medium were processed and analyzed spectrophotofluorometrically as previously described.

Biological Availability—Flumizole absorption in the rat was studied after the oral and intraperitoneal routes of administration with the polyethylene glycol 300 vehicle (I) employed in anti-inflammatory tests. Dogs received 10 mg of flumizole/kg, orally as encapsulated unformulated drug or as a solution in I (10 ml) and intravenously in 3 ml of I as a bolus or as a 3-min infusion into a rapid saline drip into the jugular vein.

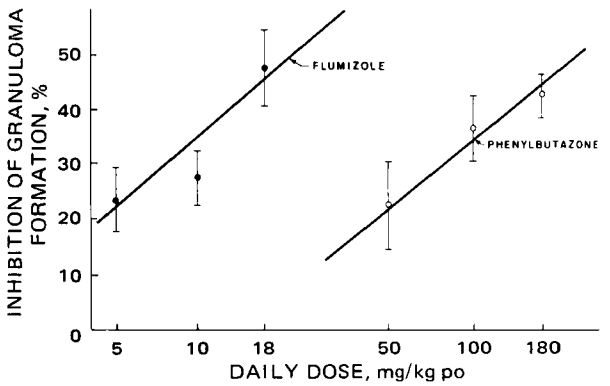


Figure 2—*Dose–response regression lines for the antigranuloma effects of flumizole and phenylbutazone in rats, five animals at each observation (mean ± SD).*

[11] Millipore, 0.22-μm pore.
[12] Parke-Davis No. 2 hard gelatin.
[13] Cab-O-Sil, Cabot Corp.

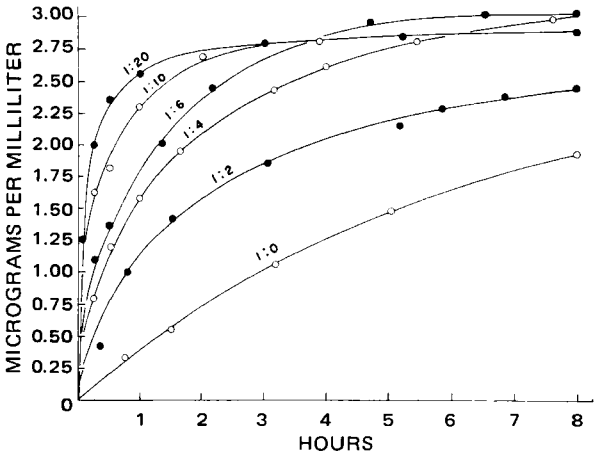


Figure 3—*Dissolution profile of flumizole–polyethylene glycol 6000 (1:x) solid dispersions in simulated gastric fluid (60 rpm, 37 ± 0.5°).*

Comparative dosage form studies with flumizole solid dispersion were conducted in two beagles fasted each night prior to drug administration at weekly intervals. Animals were allowed free access to water after drug ingestion, although food was withheld for an additional 7 hr. Drug analysis was conducted with heparinized whole blood samples drawn at selected intervals from the abdominal aorta of rats maintained under ether anesthesia or from the jugular vein of dogs. Plasma, immediately separated by centrifugation, and total urine and fecal specimens collected in metabolism cages were frozen prior to analysis.

Pharmacokinetic studies were conducted with the solid dispersion capsule formulation in healthy male volunteers during single- (25, 50, and 75 mg administered at weekly intervals) and multiple- (25 mg tid, 10 days) dose tolerance studies. Subjects were fasted overnight prior to single-dose studies and received normal noonday meals 3–4 hr later. During the multiple-dose phase, drug was administered at 4-hr intervals following an initial postprandial morning dose. Serial blood samples were collected into heparinized tubes for plasma drug level determinations.

All samples, including total urine and fecal collections from selected individuals, were frozen upon collection until analysis. Drug level analyses during availability studies were aided by computer-assisted determinations of areas under the plasma concentration–time curves (trapezoidal method). Mean levels based on area ($\bar{c}$) were obtained by dividing area by the number of hours within the time period of interest.

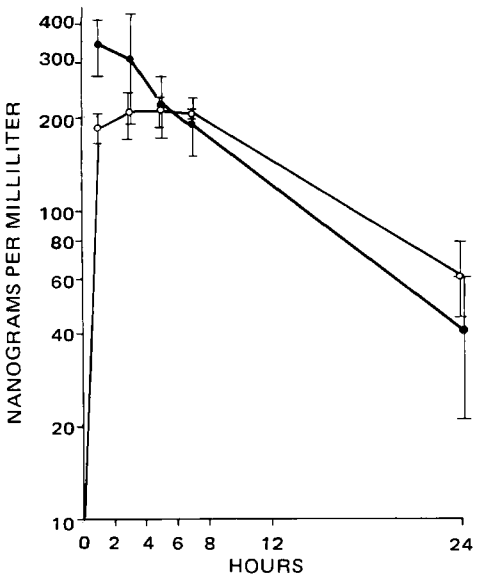


Figure 4—*Mean ± SD plasma levels of flumizole in rats (three per time interval) following intraperitoneal (●) and oral (○) administration of flumizole in polyethylene glycol 300 solution, 3.3 mg/kg.*

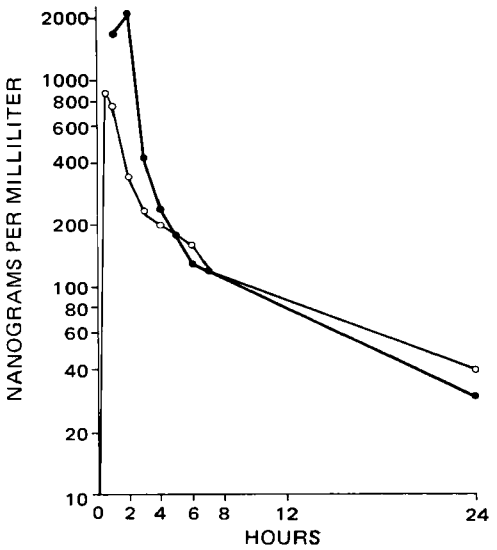


Figure 5—*Plasma levels of flumizole in a female beagle (10.6 kg) following the intravenous bolus (●) and oral (○, 4 weeks later) administration of flumizole in polyethylene glycol 300 solution, 10 mg/kg. Feces (0–48 hr) of this dog contained 43% of the oral dose but none of the intravenous dose as flumizole.*

RESULTS

Antiedema Activity—In the intact Charles River rat, flumizole consistently inhibited edema formation at oral doses of 0.3 mg/kg and above. Some variation was noted, possibly linked to the limited solubility of flumizole, even though administration was performed in the rat in aqueous polyethylene glycol 300 solution. Optimally, however, flumizole was 10 (range of 5–40) times as effective as indomethacin (Fig. 1). The activity of flumizole was not significantly reduced by adrenalectomy; at an oral dose of 1.0 mg/kg, flumizole inhibited edema formation to 54 ± 4% (*cf.*, 57 ± 7% in intact rats).

Antigranuloma Activity—In this test, flumizole possessed 10 times the potency of phenylbutazone (Fig. 2). At the highest doses, both flumizole (18 mg/kg) and phenylbutazone (180 mg/kg) slightly inhibited body weight gain during the 3-day test period.

Antierythema Activity—Flumizole and phenylbutazone both inhibited the erythema caused by UV irradiation in the guinea pig. The largely subjective nature of the evaluation of the end-point of this test (degree of erythema) precluded rigorous estimation of relative potency. Scores for both drugs were 0.8–1.1 at 1.0 mg/kg, 0.5–0.7 at 10 mg/kg, and 0.2–0.4 at 18 mg/kg; concurrently treated control animals scored 3.4–4.0. Therefore, over the 1.0–18-mg/kg dose range, the compounds were approximately equally effective.

Prostaglandin Synthetase Activity—Flumizole inhibited the conversion of arachidonic acid into prostaglandins E_2 and $F_{2\alpha}$ *in vitro* (Table I). The compound was eight times more potent than indomethacin and 470 times more potent than phenylbutazone.

Formulation—Flumizole dissolved in simulated gastric fluid from the solid dispersions containing various amounts of polyethylene glycol 6000 faster than when presented as the compound alone (Fig. 3). Solid dispersions containing increasing amounts of polyethylene glycol 6000 (2, 4, 6, 10, and 20 parts for each part of drug) showed a corresponding increase in the dissolution rate of flumizole.

The addition of lactose and 0.5% fumed silica to the solid dispersion provided a capsule formulation suitable for machine processing. This formulation displayed a dissolution rate profile in simulated gastric fluid comparable to that of the pure solid dispersion. Considerations of capsule size led to the selection of the 1:4 (w/w) formulation for clinical studies.

Biological Availability—Plasma flumizole level determinations in rats following the oral and intraperitoneal administration of flumizole, 3.3 mg/kg in polyethylene glycol 300 solution, indicated that drug was well absorbed at dose levels employed during anti-inflammatory studies (Fig. 4). Peak levels of approximately 200 ng/ml were achieved 3–7 hr after the oral dose. Following in-

Table II—Plasma Levels and Fecal Excretion of Flumizole in Two Beagles following Administration of Flumizole in Different Dosage Forms

Dosage Form	Dose Level, mg/kg	Hours after Dose										0–7-hr Area under Curve, (ng hr)/ml	Fecal Excretion, % of Dose, 0–48 hr
		0	0.25	0.5	1	2	3	5	7	10	24		
		Plasma Levels, ng/ml											
Dog 1 (female, 10.8 kg)													
Intravenous infusion	10	0	4430	—	2740	1610	650	360	190	170	70	7560	0
Oral solution	10	0	70	100	140	180	110	80	40	10	10	700	30
Oral solid drug	10	0	—[a]	0[b]	0	—	0	60	100	—	110	210	16
Oral solid dispersion	10	0	—	0	20	120	120	60	30	—	0	462	32
	50	0	—	0	70	250	220	110	90	—	40	945	68
Dog 2 (male, 17.2 kg)													
Intravenous infusion	10	0	4180	—	4270	1820	560	330	160	90	50	8785	0
Oral solution	10	0	170	360	390	250	170	50	80	10	40	1155	27
Oral solid drug	10	0	—	—	0	—	0	0	0	—	0	0	93
Oral solid dispersion	10	0	—	0	10	150	120	110	40	—	10	595	18
	50	0	—	0	160	310	150	90	0	—	0	833	88

[a] Designates levels not determined. [b] Zero levels represent less than 10 ng/ml, minimum assay detectability during this study.

traperitoneal injection, drug levels declined with an apparent half-life of 7 hr within the first 24-hr period. The absence of detectable unchanged drug in the feces and urine of these animals (oral or intraperitoneal dose) indicated the extensive (>90%) metabolism of flumizole by rats prior to excretion[14].

Preliminary studies in beagles suggested poor absorption of solid unformulated flumizole. Plasma concentrations were low, and a major portion of the dose was excreted unchanged in feces. Oral administration of drug as a solution in polyethylene glycol 300 afforded maximum plasma concentrations within 1 hr; these levels were approximately one-half those obtained following intravenous drug injection in the same animal (Fig. 5). An apparent plasma half-life of 2.5 hr during the 3–7-hr postdose period increased with time, a probable consequence of drug redistribution. The recovery of 43% of the oral dose in feces as flumizole and none following intravenous drug administration indicated that absorption was incomplete at this dose level even when drug was administered in solution. With additional evidence that dog urine (intravenous or oral dose) contained no flumizole, it could be established that absorbed flumizole was completely metabolized in this species.

To support the selection of a flumizole dosage form with optimum biological availability properties, comparative blood level studies were conducted in two beagles at successive weekly intervals (Table II). Baseline data were first obtained following the intravenous infusion and oral ingestion of polyethylene glycol 300 solutions of drug. The solid dispersion afforded maximum plasma drug levels of about 100 ng/ml 2–3 hr after the dose. The availability of flumizole from the dispersion was 50–70% that of oral solutions based on area-under-the-curve analyses. Drug levels following administration of capsules containing solid unformulated drug were appreciably lower or undetectable. At 10 mg/kg, drug in the feces of these animals (Table II) supported the incompleteness with which flumizole is orally absorbed in dogs, regardless of dosage form. A fivefold increase in dose to 50 mg/kg only doubled peak plasma levels and elevated areas under the curve 1.4–2-fold in the beagles to indicate further that limits of the drug absorption processes had been reached.

The availability of flumizole from the solid dispersion capsule formulation was assessed in humans by plasma and fecal drug level determinations following the oral administration of single and multiple doses. In 17 volunteers, drug levels peaked after 1–4 hr and averaged 20, 30, and 48 ng/ml of plasma following single doses of 25, 50, and 75 mg, respectively (Table III and Fig. 6); however, appreciable intersubject plasma level variations were evident. The disappearance of drug from plasma proceeded nonexponentially with an increasing half-life in most subjects. A shoulder, plateau, or occasional secondary peak was observed within the first 6 hr in many individuals; at the 75-mg level, 10 of 17 subjects exhibited this phenomenon, which may reflect an irregular drug absorption pattern[15]. A mean half-life of 4.4 hr was apparent during the first 6 hr in all subjects at the maximum 75-mg dose level. Based on measurable 6- and 24-hr levels at this dose in 11 subjects, a longer secondary half-life phase ranging from 4.5 to 20 hr was indicated (mean of 11 hr).

Plasma flumizole levels during a multiple-dose study were maintained without accumulation (Table III). Levels midway through the 10-day trial, 25 mg tid, averaged about 30 ng/ml. On the morning after the last multiple daily dose, drug levels had declined in all subjects to about 10 ng/ml or less. Analysis following a final single dose on Day 11 revealed the usual pattern of drug clearance with levels of less than 10 ng/ml after 24 hr.

The absence of measurable levels of unchanged drug in feces (<20% of dose) and urine (<5% of dose) from selected volunteers at the 75-mg single-dose level supported good drug absorption and indicates the completeness with which flumizole is metabolized prior to excretion by humans.

DISCUSSION

Flumizole demonstrates potent inhibitory activity in classical animal models of inflammation. The erythema formed in response to UV irradiation was inhibited. Flumizole inhibited carrageenan-induced foot edema with a potency greater than that of indomethacin. Some variation in potency was noted, it could be observed from experiment to experiment and was probably a reflection of the very limited aqueous solubility of flumizole. In addition, the potency varied in the strain of rat, flumizole being a somewhat less effective antiedema agent in the Wistar rat than in the Charles River rat (2). Full antiedema activity was retained in adrenalectomized Charles River rats, revealing that anti-inflammatory activity is not dependent upon adrenal gland stimulation. In a model of more chronic inflammation, flumizole inhibited the formation of granulation tissue around an implanted string irritant (sterile string).

Flumizole also inhibits the activity of prostaglandin synthetase *in vitro*. The drug was severalfold more potent than indomethacin and 470 times more active than phenylbutazone in a system examining prostaglandin production. Thus, despite its different physicochemical properties, flumizole resembles the acidic anti-

[14] Analysis of rat urine revealed the presence of *O*-monodemethylated flumizole by mass spectrometric analysis of material isolated from thin-layer plates. Subsequent confirmation of this finding and the detailed elaboration of flumizole metabolism pathways will be the subjects of a forthcoming paper.

[15] Noonday meals were generally taken during a period coincident with the observed drug level fluctuations. Similar blood level patterns reported with the anti-inflammatory agent indoxole (8) may have been related to food intake and consequent gallbladder evacuation of drug. Although double peaking was observed with flumizole in dogs (three of six experiments, intravenous dose), a biliary fistula beagle that received an oral dose of ^{14}C-flumizole secreted very little (by chromatographic estimation) unchanged drug with bile.

Table III—Mean Plasma Levels of Flumizole in Humans during Single- and Multiple-Dose Tolerance Trials following Administration of Flumizole Solid Dispersion Capsule Formulation

Single-Dose Studies, 17 Subjects

Dose, mg (mg/kg, Mean)	Mean Plasma Drug Levels, ng/ml	
	Peak (Range)	$\bar{c}$, 0—6 hr
25 (0.33)	20 ± 11 (7—36)	11 ± 6
50 (0.67)	30 ± 17 (7—66)	17.5 ± 7
75 (1.0)	48 ± 18 (19—87)	28.5 ± 9

Multiple-Dose Studies, Nine Subjects

	Mean Plasma Drug Levels, ng/ml (Range)[a]								
	Day 1			Day 4		Day 11 (Single am Dose)			
Dose	0 hr	4 hr	6 hr	4 hr	6 hr	0 hr	2 hr	6 hr	24 hr
25 mg tid	5 ±3 (0—8)	22 ±21 (6—74)	25 ±20 (3—68)	26 ±12 (11—46)	31 ±16 (6—56)	8 ±3 (4—13)	26 ±14 (9—54)	17 ±14 (3—50)	3 ±3 (0—7)

[a] Hourly intervals designated refer to time period after the first dose of the day; drug was administered at 8:00 am, noon, and 4:00 pm daily. This 10-day study culminated a 25-day trial, in which the dose increased incrementally from 5 mg bid to 15 mg tid through the first 15-day segment.

inflammatory agents in its inhibition of prostaglandin synthetase. The demonstration of anti-inflammatory activity for flumizole in humans[16] is further support for the hypothesized importance of this activity in the mechanism of action of anti-inflammatory agents (9).

Because the aqueous solubility of flumizole is very low, all pharmacological testing was conducted using a solution of the drug in polyethylene glycol 300 containing polysorbate 80. With the development of sensitive fluorometric assay procedures specific for flumizole, measurement of plasma drug concentrations confirmed the absorption of drug from this vehicle in dogs (10 mg/kg) and rats (3.3 mg/kg). For extended studies in animals and humans, a solid dosage form of flumizole was sought with optimum absorption characteristics.

The dissolution of a poorly water-soluble drug in a melt of the water-soluble carrier polyethylene glycol 6000, followed by rapid cooling, affords a solid in which drug particles are finely dispersed, with resultant improvements in wetting properties of the drug (10). The dissolution rates of griseofulvin (11) and indomethacin (12) and the absorption of griseofulvin in dogs (13) and humans (14) have been improved using polyethylene glycol 6000 dispersion systems. The preparation of a solid dispersion of flumizole with polyethylene glycol 6000 (1:4, w/w) by the melting method provided an oral dosage form with an increased dissolution rate in simulated gastric fluid and improved plasma drug concentration patterns in beagles. Plasma levels were higher and were more rapidly and uniformly achieved than from an unformulated dose; solid dispersion drug levels more closely resembled those following the administration of oral solutions of drug. The amounts of unabsorbed drug in feces generally supported these findings. Therefore, the solid dispersion of flumizole was selected as a superior dosage form for further investigation.

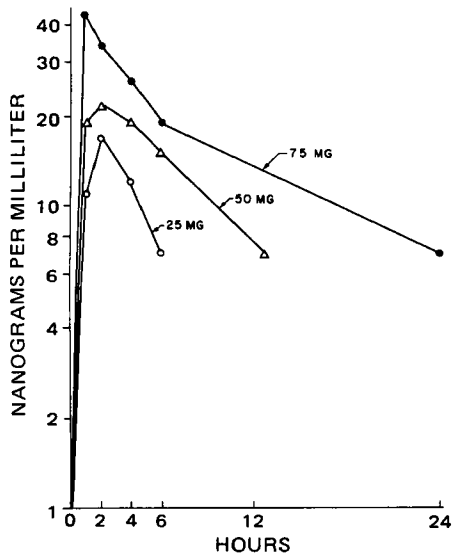


Figure 6—*Mean plasma levels of flumizole in 17 subjects following the administration of single doses of 25 (○), 50 (△), and 75 (●) mg of flumizole solid dispersion capsule formulation.*

Following preclinical safety evaluation in animals[17], bioavailability studies were conducted in humans with the solid dispersion capsule formulation of flumizole. A dose-dependent increase in mean plasma levels of flumizole was observed over the single-dose range of 25–75 mg. Peak plasma levels of flumizole were achieved with 1–4 hr after the dose was received. The magnitude of plasma levels and the absence of appreciable quantities of drug in feces supported good drug absorption during these studies. Similar drug level patterns were observed during multiple-dose regimens of 25 mg tid.

Flumizole was eliminated from the bloodstream of rats (3.3 mg/kg ip) with a plasma half-life of about 7 hr within the first 24-hr period. In dogs (10 mg/kg po and iv) and humans (75 mg po), plasma flumizole levels declined initially with an apparent half-life of 2–4 hr and increased with time, a probable consequence of drug redistribution. Detailed pharmacokinetic properties of flumizole will be discussed in a future publication.

The present studies demonstrate flumizole to be a potent anti-inflammatory agent in standard rodent and prostaglandin synthetase systems. Biological availability of this poorly water-soluble compound is optimized in dogs and humans by administration as a solid dispersion with polyethylene glycol 6000.

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* Present address: Pharmaceutical Research and Development, Burroughs Wellcome & Co., Greenville, NC 27834

ˣ To whom inquiries should be directed.

Apparent Absorption Kinetics of Micronized Griseofulvin after Its Oral Administration on Single- and Multiple-Dose Regimens to Rats as a Corn Oil-in-Water Emulsion and Aqueous Suspension

THEODORE R. BATES ˣ and PHILIP J. CARRIGAN

Abstract □ This investigation was designed to quantitate and compare in the rat the oral absorption characteristics of micronized griseofulvin from a corn oil-in-water emulsion dosage form containing suspended drug and a control aqueous suspension after single-dose (50 mg/kg) and multiple-dose (50 mg/kg every 12 hr for five doses) administrations. The time course of intact drug in the plasma of all animals was best described by a one-compartment open model with apparent zero-order absorption. In contrast to that observed with the aqueous suspension, the onset of drug absorption after single-dose administration of the corn oil emulsion was significantly delayed. This difference disappeared upon multiple dosing of the two dosage forms, with the mean onset being quite rapid in both cases. Administration of a single dose of the antibiotic as the corn oil emulsion resulted in considerable increases in the maximum plasma levels of griseofulvin and in the duration, relative extent, and uniformity of drug absorption compared to those observed after administration of the control aqueous suspension. The potentiating effects of the lipid on drug absorption persisted on multiple dosing but at a somewhat reduced level.

Keyphrases □ Griseofulvin, micronized, absorption kinetics—corn oil-in-water emulsion compared to aqueous suspension, single and multiple doses, rats □ Absorption kinetics, micronized griseofulvin—corn oil-in-water emulsion compared to aqueous suspension, single and multiple doses, rats □ Corn oil-in-water emulsion, micronized griseofulvin—absorption kinetics compared to aqueous suspension, single and multiple doses, rats

Several investigators (1–3) suggested that the slow rate and low extent of absorption, as well as the appreciable intersubject differences in the amount of micronized griseofulvin absorbed from conventional dosage forms, are the most common reasons for clinical failure with griseofulvin therapy. In a previous report (4), it was demonstrated, using the rat as an animal model, that the maximum plasma levels, the bioavailability, and, of considerable importance, the uniformity of absorption of micronized griseofulvin were markedly enhanced after oral administration of a single dose of the antibiotic dispersed in a corn oil-in-water emulsion dosage form vehicle.

These findings were recently confirmed in a study conducted in humans in which the absorption characteristics of micronized griseofulvin from the same corn oil-in-water emulsion were compared with those from an aqueous suspension and two different commercial tablet dosage forms (5). The results of this study provided evidence that the maximum plasma levels and bioavailability of micronized griseofulvin can be increased approximately three- to fourfold and twofold, respectively, by administering the drug as a corn oil-in-water emulsion.

It is not without precedence for absorption differences, which appear after *single-dose* drug administration, to diminish or even to disappear upon *multiple* oral dosing of a drug or drug product (6). Hence, the purposes of the present investigation were to assess and to compare the absorption profiles of micronized griseofulvin from a corn oil-in-water emulsion and a control aqueous suspension dosage form after single and repetitive administrations to rats. The resultant plasma antibiotic level–time data were subjected to pharmacokinetic analysis with the intent of developing a mechanistic explanation for the