Enhancement of Bioavailability of a Hydrophobic Amine Antimalarial by Formulation with Oleic Acid in a Soft Gelatin Capsule

V. STELLA *, J. HASLAM ‡x, N. YATA *§,
H. OKADA *¶, S. LINDENBAUM *, and T. HIGUCHI *‡

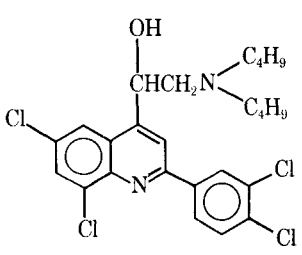
Received November 30, 1977, from the *Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045, and the ‡INTERx Research Corporation, Lawrence, KS 66044. Accepted for publication January 30, 1978. §Present address: Faculty of Pharmaceutical Sciences, Osaka University, Osaka, Japan. ¶Present address: Takeda Chemical Industries, Osaka, Japan.

Abstract □ The relative availability of the orally administered hydrophobic antimalarial α-(dibutylaminomethyl)-6,8-dichloro-2-(3′,4′-dichlorophenyl)-4-quinolinemethanol (I) from two dosage forms was determined in beagle dogs. Compound I was soluble in oleic acid to the extent of 23.5% (w/w), and oleic acid was suitable for encapsulation in soft gelatin capsules. The availability of I formulated as its hydrochloride salt in a standard hard gelatin capsule formulation was significantly lower than that of I formulated in a soft gelatin capsule with oleic acid as the solvent. A 20% solution of I in oleic acid (soft gelatin capsules) maintained at 23° provided 4% of the oleic acid ester of I within 1 month. Further reaction, however, was not seen over 2 years.

Keyphrases □ α-(Dibutylaminomethyl)-6,8-dichloro-2-(3′,4′-dichlorophenyl)-4-quinolinemethanol—bioavailability from two dosage forms compared, dogs □ Bioavailability—quinolinemethanol antimalarial from two dosage forms compared, dogs □ Antimalarials—α-(dibutylaminomethyl)-6,8-dichloro-2-(3′,4′-dichlorophenyl)-4-quinolinemethanol, bioavailability from two dosage forms compared, dogs

The antimalarial compound α-(dibutylaminomethyl)-6,8-dichloro-2-(3′,4′-dichlorophenyl)-4-quinolinemethanol[1] (I) as its hydrochloride salt was effective in both animals and humans against chloroquinone-resistant strains of malaria (1–4). Because of the extremely low solubility of I (5), leading to poor bioavailability, large doses of the drug are required to achieve effective blood levels. Typical dosage regimens required to obtain a significant therapeutic effect consisted of 250 mg of the hydrochloride in a hard gelatin capsule formulation[2] administered orally three times a day for 6 days. The large recovery of I in fecal matter from rats given I hydrochloride orally (6) and its low aqueous solubility suggested that the bioavailability of I from its current dosage form was probably poor.

Attempts to improve the bioavailability of I by preparing more water-soluble salt forms, a method successfully employed previously to enhance the water solubility of a hydrophobic amine antimalarial drug (7), were unsuccessful[3]. This paper reports a promising formulation of I utilizing oleic acid as a solvent in a soft gelatin capsule.



I

EXPERIMENTAL

Materials—The free base was prepared by treating a methanol solution of I hydrochloride with sodium hydroxide. The precipitated free base was filtered and dried at 50° under vacuum. Oleic acid was obtained commercially[4].

Aqueous Solubility of I Hydrochloride—Excess I hydrochloride was agitated in a thermostated dissolution apparatus at 25° in water for varying times. A portion of the supernate was filtered, and the absorbance was measured[5] at 272 nm. The hydrochloride solubility was approximately 1 mg/liter. Very poor reproducibility was obtained in the solubility measurements because I tended to plate out on the walls of the dissolution apparatus and spectrophotometer cells (8), and the solid undissolved material recovered from the aqueous suspension was not the hydrochloride but the free base. Subsequent determination of the aqueous solubility of I showed it to be also approximately 1 mg/liter.

Formulation and Stability of I in an Oleic Acid Solvent—Compound I in oleic acid was soluble to the extent of 23.5% (w/w). Hand-filled soft gelatin capsules[6] were prepared using a 20% (w/w) solution of I in oleic acid. The capsules were weighed before and after injecting the liquid formulation so that the dose contained in each capsule could be determined. Each capsule could hold about 80 mg of I. These hand-filled capsules were prepared just before dosing of the dogs.

A stability study of I in oleic acid was carried out on a small batch of the soft gelatin capsule formulation[7]. At temperatures of 40° and higher, three degradation products were observed with TLC; however, at 23° over 2 years, only one of these degradation products was observed. This product was the ester formed between oleic acid and I. Analysis by high-pressure liquid chromatography (HPLC) showed that about 4% of this ester had formed in a short period after preparation of a small batch of capsules (1 month), but analysis at 1 and 2 years showed no further increase in the amount of the ester formed.

Standard Gelatin Capsule Formulation—Hard gelatin capsules containing 250 mg of I as its hydrochloride in a solid formulation were obtained[7]. The capsules were the standard formulation of I hydrochloride used in a number of clinical trials. Analysis for I in the capsules showed them to contain the labeled amount of I as its hydrochloride.

***In Vivo* Absorption Studies**—Four female (Dogs 1–4 in Fig. 1) and one male (Dog 5 in Fig. 1) beagle dogs received either 250 mg of I as its hydrochloride (equivalent to 234 mg of I) in the standard hard gelatin capsule or 234 mg of I in a total of three oleic acid soft gelatin hand-filled capsules.

The dogs were fasted overnight prior to dosing. After administration of either the soft or hard gelatin capsule, 200 ml of water was given through a gastric tube. The dogs were allowed water *ad libitum* during the study. At predetermined times, 5-ml blood samples were removed and assayed for I by HPLC. After a 3-week lapse, the dogs were given the other dosage form.

Assay of I in Blood—The 5-ml blood samples were discharged immediately into 15-ml glass (polytef-lined screw-capped) centrifuge tubes. The blood was mixed with 2 drops of 15% ethylenediaminetetraacetic

[1] The Walter Reed designation for the hydrochloride salt of this compound is WR30090. This compound is currently undergoing testing by the Walter Reed Army Medical Center. Compound I hydrochloride was obtained from the Walter Reed Army Medical Center under Contract DADA17-73-C-3125.

[2] Control No. E-310, Lafayette Pharmacal.

[3] Unpublished results.

[4] Emersol 633LL, Emery Industries.

[5] Cary model 15 recording spectrophotometer, Varian Associates.

[6] Red oblong capsules (9.5 minims).

[7] A batch of 1000 capsules was prepared at Banner Gelatin Products Corp., Chatsworth, Calif.

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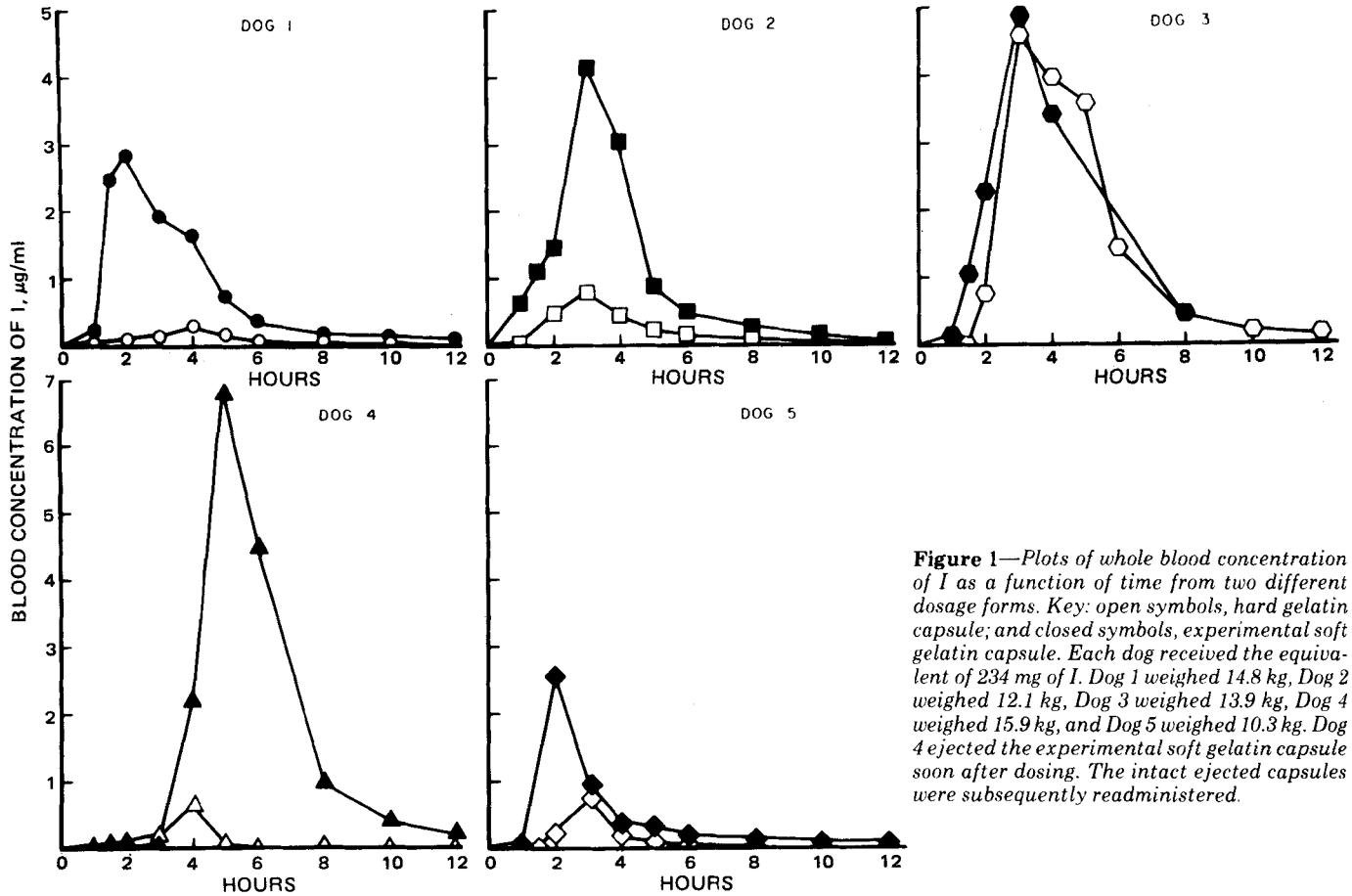

Figure 1—*Plots of whole blood concentration of I as a function of time from two different dosage forms. Key: open symbols, hard gelatin capsule; and closed symbols, experimental soft gelatin capsule. Each dog received the equivalent of 234 mg of I. Dog 1 weighed 14.8 kg, Dog 2 weighed 12.1 kg, Dog 3 weighed 13.9 kg, Dog 4 weighed 15.9 kg, and Dog 5 weighed 10.3 kg. Dog 4 ejected the experimental soft gelatin capsule soon after dosing. The intact ejected capsules were subsequently readministered.*

acid solution previously added to the centrifuge tube. Reagent grade ether, 5 ml, was then added to the centrifuge tube, which was shaken vigorously on a wrist-action shaker with modified, 17.8-cm long rocker arms to hold the centrifuge tubes horizontally.

After 1 hr of continuous shaking, the tubes were removed and centrifuged for 10 min at approximately 5000×*g*. The centrifuge tubes were then immersed in a dry ice–acetone bath to freeze the aqueous layer. The ether layer, about 4 ml, was decanted into a 15-ml conical glass centrifuge tube. The extraction was repeated using the same procedure. Then the ether layer from the second extraction (about 5 ml) was added to the ether from the first extraction, and both were evaporated at room temperature to about one-third of the original volume[8].

The extraction procedure was repeated a third time, and the ether layer (5 ml) was added to the ether from the previous extraction steps. The total ether extracts then were evaporated under vacuum, one tube at a time, by tipping the tube nearly horizontal to prevent bumping. The centrifuge tubes were then placed in a vacuum desiccator over calcium chloride, and the contents were dried completely overnight under vacuum in the dark. Okada *et al.* (9) noted the rapid photolytic degradation of I in organic solvents. The solid material remaining from the ether extracts was dissolved, just prior to analysis, in 100 μl of 20% chloroform–80% heptane (all chemicals were standard reagent grade). Usually 5 or 10 μl of this solution was then injected onto the HPLC column.

A high-pressure liquid chromatograph[9] equipped with a 280-nm UV detector was used. The column was 1.8 mm i.d. stainless steel, 50 cm long, with a silica stationary phase[10].

Several different mobile phases were used, without success, to find conditions yielding a good separation of I free base from the other blood components for as many as 400 injections. However, a system was developed in which one column could be used for 400 injections; but after approximately 40 injections, the composition of the mobile phase needed to be changed slightly to retain good peak separation.

The mobile phase used with a fresh column was 10% (v/v) methanol in the stock solvent [20% (v/v) dioxane–heptane]. The mobile phase was dried over anhydrous sodium sulfate and then degassed to achieve reproducible results. As the column was used, the separation of I from the blood components became poorer and the amount of methanol was decreased to give a good separation. The sensitivity also decreased. A column was used until the methanol concentration was decreased to just less than 3%. A standard concentration of I in 20% (v/v) chloroform–heptane was injected before and after each blood sample as the external standard. The average area of the standard peaks was used to define the column sensitivity for that particular run.

The recovery of I from spiked blood samples was determined using this extraction procedure. The recovery of I was 80% for samples containing 20 ng or more of I in 5 ml of whole blood.

RESULTS AND DISCUSSION

The potential for utilizing a carboxylic acid as a solvent for preparing a solution dosage form of I was recognized when I was found to be extremely soluble in butyric acid and other low molecular weight carboxylic acids. Because of its low volatility and physiological compatibility, oleic acid was chosen as the solvent for a soft gelatin formulation of I. As stated under *Experimental*, I was soluble in oleic acid to the extent of 23.5% (w/w) and formed only a small amount of a degradation product over 2 years of storage at room temperature.

The degradation product was shown by TLC[11] to be the oleic acid ester of I. The identity was confirmed by synthesizing an authentic sample of the ester and comparing its chromatographic properties to those of the formed product. The ester formed to the extent of 4% in 1 month and maintained that level for up to 2 years at 23°. The fact than an equilibrium appeared to be reached could be explained by one of two mechanisms. The product may have formed due to small quantities of oleic anhydride or other reactive species originally present in the oleic acid. Or the esterification may have been simply due to the reaction of I with oleic acid to form the ester and a corresponding amount of water and this reaction rapidly established an equilibrium. Experiments carried out to determine which mechanism applied were inconclusive.

[8] Rotary Evapo-Mix (test tube model).
[9] Model 4000, Varian Associates.
[10] Corasil II, Waters Associates.

[11] Analtech silica gel 250 GF plates, developed from the upper layer of hexane–acetone–ammonium hydroxide (80:14:6) and detected by a UV lamp (254 nm).

Table I—Twelve-Hour Area under the Blood Level *versus* Time Curve (AUC_0^{12}) Comparison for the Hard *versus* Soft Gelatin Capsule Formulations of I

Number	AUC_0^{12} (Hard Gelatin Capsule), μg hr/ml	AUC_0^{12} (Soft Gelatin Capsules), μg hr/ml	$\frac{AUC_0^{12} \text{ (Hard Gelatin Capsule)}}{AUC_0^{12} \text{ (Soft Gelatin Capsules)}}$
1	0.99	9.02	0.11
2	2.46	12.18	0.20
3	16.53	17.53	0.94
4	0.94	18.96	0.05
5	1.38	5.19	0.27
$\bar{x} \pm SD$	4.46 ± 6.77	12.58 ± 5.76 ($p < 0.05$)[b]	0.31 ± 0.36
$\bar{x} \pm SD$[a]	1.44 ± 0.71[a]	11.33 ± 5.83[a] ($p < 0.01$)[b]	0.16 ± 0.10[a]

[a] Numbers refer to the mean and standard deviation values if the data in each column for Dog 3 are disregarded. The AUC_0^{12} for Dog 3 given the hard gelatin capsule could be rejected using the Q test. [b] The AUC_0^{12} for the soft gelatin capsule formulation is significantly different from the AUC_0^{12} at the level indicated using the Student t test.

The results of the blood level–time studies for each dog for the two formulations are given in Fig. 1. Table I summarizes the area under the curve (AUC) data for the blood level–time profile. Also included is the relative AUC for the hard gelatin capsule *versus* the soft gelatin capsule formulation. Compound I was delivered significantly more efficiently from the oleic acid solvent soft gelatin capsule than from the hard gelatin capsule. For both formulations, there appears to be a significant lag time of 1–3 hr for I absorption.

The oleic acid formulation of the hydrophobic drug, I, in a soft gelatin capsule allowed the poorly water-soluble drug to enter the GI tract in a readily dispersible form, providing for more rapid and complete drug absorption. The mechanism for the increased I bioavailability can be postulated to be the emulsification of the oleic acid by the GI contents, resulting in the release of I which is then rapidly absorbed. Whether the drug is carried along by oleic acid absorption or whether it is actually released from oleic acid and subsequently absorbed is not known.

In summary, it appears that I as its hydrochloride is poorly bioavailable from its standard hard gelatin capsule formulation relative to I formulated in a soft gelatin capsule utilizing oleic acid as a solvent. The poor bioavailability of I from the hard gelatin capsule is probably due to its low aqueous solubility combined with a poor formulation design.

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ACKNOWLEDGMENTS

Supported by Contract DADA17-73-C-3125, Department of the Army, U.S. Army Medical Research and Development Command (Paper 1490 in the Malarial Publication Series) and by National Institutes of Health Grant GM22357.

Heterocyclic Tricycles as Potential CNS Agents I: 4-Aminoalkylindeno[1,2-*c*]pyrazoles

THOMAS L. LEMKE *[x], MICHAEL B. CRAMER [‡], and K. SHANMUGAM *

Received November 7, 1977, from the **Department of Medicinal Chemistry and Pharmacognosy and the* [‡]*Department of Pharmacology, College of Pharmacy, University of Houston, Houston, TX 77004.* Accepted for publication January 31, 1978.

Abstract □ Series of 4-(3-dimethylaminopropyl)-4-hydroxyindeno[1,2-*c*]pyrazoles and 4-(1-methyl-4-piperidyl)-4-hydroxyindeno[1,2-*c*]pyrazoles were synthesized and identified. The compounds were evaluated as potential CNS agents using spontaneous and forced motor activity in mice as an initial test. 2-Ethyl-3-methyl-4-(1-methyl-4-piperidyl)-4-hydroxyindeno[1,2-*c*]pyrazole possessed significant biological activity.

Keyphrases □ Indenopyrazoles, substituted—synthesized, evaluated for CNS activity in mice □ CNS activity—evaluated in substituted indenopyrazoles in mice □ Structure–activity relationships—substituted indenopyrazoles evaluated for CNS activity in mice □ Heterocycles, tricyclic—substituted indenopyrazoles synthesized, evaluated for CNS activity in mice

Since the introduction of chlorpromazine, a potent central nervous system (CNS) depressant of the phenothiazine class, numerous tricyclic analogs have been investigated. Modification of the side chain and the central ring of the phenothiazines resulted in discovery of additional CNS depressants as well as CNS stimulants. A limited number of investigations involved replacement of one of the benzene rings with heterocyclic aromatic rings. The syntheses of 4-(3-dimethylaminopropylidene)-9,10-dihydro-4*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophene (I) derivatives (1) and 2-methyl-4-(3′-dimethylaminopropylidene)-9,10-dihydro-4*H*-benzo[5,6]cyclo-