In summary, our results have shown that the gastric absorption of captopril would contribute to the early appearance of captopril in the blood, but the intestine has a greater capacity than the stomach for the absorption of captopril and the relative magnitude of captopril absorption from the gastric site will depend on the transit rate through the stomach. Furthermore, it has been shown that chronic treatment of captopril in the rat does not increase the rate of captopril absorption from either the stomach or intestine. In comparison to salicylic acid, captopril is not as rapidly absorbed from either the stomach or intestine.

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Pharmacokinetics of Probucol in Male Rats

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Abstract □ The bioavailability and pharmacokinetic behavior of 10 mg/kg of [^{14}C]probucol in an oil-water emulsion was determined after oral and intravenous administration to rats. The bioavailability of the oral formulation was ~6%. For the first 12-h interval after the intravenous bolus, plasma probucol concentrations increased after an initial decrease. This effect may be attributed to the formulation or precipitation of the drug in the vasculature. The terminal plasma half-life was 6 d. By 7 d, 45 and 4.65% of the labeled intravenous bolus was excreted in the feces and in the urine, respectively. Although most of the labeled dose was excreted in the bile, any enterohepatic recirculation that did occur did not contribute to the atypical plasma concentration *versus* time profile. The tissue distribution of the label and elimination rates in the bile differed between the two routes of administration. Either the total body burden, precipitation of the drug, or the emulsion vehicle may be responsible for the nonlinear distribution and clearance of the intravenous dose.

Keyphrases □ Bioavailability—oil-in-water emulsion, tissue distribution, probucol, biliary elimination, rats □ Probucol—bioavailability, oil-in-water emulsion, tissue distribution, biliary elimination

Probucol (I)[1], 4,4′-[(1-methylethylidene)bis(thio)]-bis[2,6-bis(1,1-dimethylethyl)]phenol, is an orally effective hypocholesterolemic drug. Its efficacy has been demonstrated in mice, rats, monkeys, and humans (1), and its chemical structure is unlike that of other hypocholesterolemic agents. There are no published pharmacokinetic studies of probucol in rats to characterize the dose-response of the drug (2) and only recently have aspects of the pharmacokinetic behavior of probucol in humans been presented (3).

The present pharmacokinetic studies were conducted in rats. Long-term toxicity and efficacy studies have been conducted in rats and other rodent and nonrodent species, and the identity of pharmacokinetic parameters that might assist interpretation of the outcome of the dose-response studies was sought.

EXPERIMENTAL SECTION

Probucol Formulations—[Ring(U)-^{14}C]probucol[2] with a specific activity of 7.58 or 34.1 μCi/mg was used for distribution and excretion studies. The compound is labeled with statistical uniformity in all positions of each ring. A radiochemical purity of >98% was determined with TLC, autoradiography, and liquid scintillation counting (LSC) techniques. Two TLC systems were used: hexane–benzene (80:20) and hexane–ether (95:5) on silica gel (0.25 mm) plates[3]. Uniformly ring-labeled [^{14}C]probucol with a specific activity of 34.1 μCi/mg and a radiochemical purity of 99.5% by HPLC and LSC techniques was used for the biliary excretion studies. Labeled probucol was mixed with nonlabeled probucol which had a chemical purity of >99.5% to provide the intended regimens of oral and intravenous probucol. The specific activities of the resultant mixtures ranged from 1.57 to 8.74 μCi/mg.

Both oral and intravenous formulations of probucol were prepared as oil-

[1] Merrell Dow Research Institute-Cincinnati Center. Available in the U.S. under the trademark Lorelco.

[2] Synthesized by D. F. Gransden and G. A. Roth, The Dow Chemical Co., Midland, Mich.

[3] Silica Gel, F-254; MCB Manufacturing Chemists, Inc., Cincinnati, Ohio.

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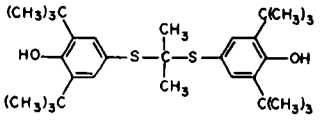

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water emulsions. Probucol was dissolved in a saturated triglyceride from coconut oil[4]. The oil-water emulsion, like the physiologically compatible hyperalimentation emulsions, was then formulated to contain 4% glucose, 1.2% vegetable lecithin, 0.3% nonionic surfactant[5], and 15% of the saturated triglyceride containing the probucol. The mixture was sonicated[6] to form the emulsion. Because of the poor aqueous solubility of probucol, coconut oil or the above emulsion was used in previous pharmacological, toxicological, and clinical studies and in the studies reported here. All dose solutions were stored under nitrogen gas to prevent autoxidation.

Animal Maintenance and Posology—Adult male Sprague–Dawley rats were individually housed in modified, Roth-type glass metabolism chambers[7] for the separate collection of urine, feces, and expired air, or were housed in stainless-steel cages prior to sacrifice. Room air was drawn through the chambers (500–600 mL/min) and then scrubbed with a solution of ethanolamine–2-methoxyethanol (3:7) to trap any $^{14}CO_2$ as the carbamate. The rooms were temperature- and humidity-controlled and operated on a 12-h light/dark cycle. Water was provided *ad libitum* and food was withheld from the animals 4 h prior to and 4 h after dosage.

Two groups of four male rats were given a single 10-mg/kg dose of [^{14}C]probucol as an emulsion to determine the tissue distribution and the rates and routes of excretion. One group was given ~1.4 mL of the emulsion orally through stainless-steel feeding needles. The other group of rats was given ~0.3 mL iv of the emulsion over a 30-s period. The intravenous emulsion was injected into a jugular vein cannula implanted 48 h earlier. After the dose was injected, the cannula was flushed with 0.2 mL of saline.

The plasma distribution and clearance of 10 mg/kg of probucol or [^{14}C]-probucol was determined in two groups of male Sprague–Dawley rats. One group of 102 rats was given a 10-mg/kg iv dose of an 8.33-mg/mL emulsion (0.12 mL/100 g body weight) by way of a jugular cannula and a second group of 75 rats was given a single oral dose of a 1.67-mg/mL emulsion (0.6 mL/100 g body weight).

Biliary excretion studies were conducted with 11 adult male Sprague–Dawley rats. A cannula of polyethylene tubing[8] was inserted into the common bile duct, anterior to the pancreatic junction, passed subcutaneously, and exteriorized dorsally. Bile was allowed to flow continuously over a 3-d recovery period. On the second day after surgery, a jugular vein cannula was placed in six of the animals. On the third day a 10-mg/kg dose of [^{14}C]probucol emulsion was given by intravenous infusion, as described previously, to six of the rats and by oral gavage to the remaining five rats.

Sample Collection—To collect an adequate volume of blood for probucol analysis, rats were decapitated and exsanguinated at predetermined times. Following oral administration blood was collected at 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 18, 24, 30, 36, 42, 48, 60, 72, 96, 120, 144, 192, 240, 288, 336, 384, 432, and 480 h postdose. Blood taken following intravenous dosing was similar except no samples were taken at 30 and 480 h and additional samples were taken at 168 h. Blood was taken from the jugular cannula or by decapitation for the biliary excretion studies. All samples were collected in heparinized containers, and the plasma fraction was stored frozen until analysis. Probucol had been shown to be stable for >16 months in frozen plasma. All analyses were completed within that time.

Urine was collected in dry ice traps, and both urine and feces were obtained every 6, 12, or 24 h and stored frozen until label analysis. The CO_2 trapping solution was changed frequently over the first 48-h test period. Tissues were harvested immediately after exsanguination and analyzed for concentrations of label activity or probucol. Bile was collected at 6-h intervals for 24 h after administration.

Sample Preparation for Probucol Analysis—*Plasma*—Plasma samples for the determination of probucol concentrations from 0.25 to 100 μg/mL were assayed by EC/GC or by HPLC and concentrations from 0.005 to 1.0 μg/mL were determined by GC/MS. All samples were fortified with aliquots of a methanolic solution containing the internal standard, 4,4′-[(1-methylbutylidene)bis(thio)]bis[2,6-bis(1,1-dimethylethyl)phenol], and the plasma protein was precipitated with methanol–acetone (3:2, v/v). After centrifugation, the liquid phase was extracted with hexane. Hexane extracts destined for HPLC were evaporated with nitrogen gas and reconstituted in methanol.

The hexane extracts destined for GC/MS were evaporated to dryness under nitrogen, reconstituted with benzene, and passed through a silica column[9]. The benzene eluant containing the probucol was evaporated to dryness under nitrogen, and the sample was reconstituted in methanol. Standard curves were prepared for all plasma samples by fortifying blank plasma with appropriate amounts of methanolic solutions of probucol prior to the above extraction schemes.

Tissue Samples—Tissues were homogenized[10] in ethanol–acetone (3:2, v/v) and diluted to 10 mL with ethanol–acetone. A 1-mL aliquot was transferred to a screw-cap test tube, and the internal standard was added along with 1 mL of hexane and 0.5 mL of water. After mixing and centrifugation, the hexane layer was evaporated to dryness with a nitrogen gas stream and the residue was reconstituted in methanol. Standard curves were prepared for tissue samples as described for plasma samples.

Instrumentation—*Electron-Capture Gas Chromatography*—The hexane extract of plasma destined for EC/GC[11] was injected directly onto a coiled glass GC column[12]. Operating conditions were as follows: carrier gas, 5% methane in argon; flow rate, 40 mL/min; injection port, 300°C; detector, 300°C; column, 270°C.

Gas Chromatography/Mass Spectrometry—The methanol extract of plasma destined for GC/MS[13] was injected onto a glass GC column[14]. Helium carrier gas at a flow rate of 20 mL/min was used. Temperatures were set as follows: injection port, 250°C; column, 280°C. The mass spectrometer was operated in the electron-impact mode. The fragment ions at *m/z* 278 (probucol) and *m/z* 306 (internal standard) were recorded by selective-ion monitoring.

Liquid Chromatography—A modular liquid chromatographic system was used in the analysis of probucol in plasma or tissue. The methanolic extracts were injected onto a reverse-phase column[15]. The HPLC system consisted of an autosampler[16], a high-pressure pump[17], and a fixed-wavelength UV (254 nm) detector[18]. The mobile phase consisted of acetonitrile, hexane, and 0.1 M ammonium acetate (900:35:65). The flow rate was 0.9 mL/min.

Analytical Recovery and Precision—The EC/GC procedure had a relative recovery of 98.4% and a relative precision at the 95% confidence level (C.L.) of 14.1%. The relative recovery of the HPLC assay was 97.2% and the relative precision was 9.6% (95% C.L.). For the lower concentrations of probucol determined by GC/MS, the relative recovery was 105.5% with a relative precision of 24% at the 95% C.L.

Sample Preparation for Radioactivity Analysis—The label activity in most of the samples was determined by direct LSC techniques. Plasma samples were prepared by the addition of 0.03–1.0 g of plasma to 0–1 mL of water; urine samples and cage washings were prepared by the addition of 0.5 mL of sample to 1 mL of water; and bile was prepared by the addition of 50 mg of bile to 1 mL of water. Ten milliliters of scintillation reagent[19,20] was then added to each sample. Exhaled $^{14}CO_2$ was trapped as the carbamate. Five milliliters of the trapping solution was then combined with 5 mL of a scintillation cocktail consisting of 120 mL of scintillation reagent[21], 220 mL of 2-methoxyethanol, and 660 mL of toluene.

Tissues were frozen in liquid nitrogen and pulverized by an electromagnetic, cryogenic impact grinding apparatus[22] (4). Samples (30–100 mg) of the pulverized tissues were added in triplicate to LSC vials containing 12 mL of scintillation fluid. A fine suspension was formed by the addition of 3 mL of water and shaking to form a sol–gel. The label activity in the samples was determined in the liquid scintillation counter[23]. External standardization was used to determine quench correction.

Aqueous fecal homogenates, equivalent to 150 mg of fecal solids, were oxidized by combustion[24] to CO_2 and trapped as the carbamate in 8 mL of a 30% solution of ethanolamine in 2-methoxyethanol. This solution was combined with 10 mL of the scintillation cocktail described earlier. Skin

[4] Noebec M-5; PVO International Inc., Boonton, N.J.
[5] Pluronic F-68; BASF Wyandotte, Wyandotte, Mich.
[6] Sonifier, Ultrasonic Cell Disruptor; Bronson Instruments, Inc., Danbury, Conn.
[7] Wyse Glass Specialties, Freeland, Mich.
[8] PE-10; Intramedic, Clay-Adams, Div. of Becton, Dickinson and Co., Parsipanny, N.J.
[9] Florisil; Fisher Scientific Co., Pittsburgh, Pa.
[10] Polytron Homogenizer; Brinkmann Instruments, Westbury, N.Y.
[11] Model 5702A equipped with a ^{63}Ni electron-capture detector; Hewlett-Packard, Avondale, Pa.
[12] 3% SP-2100 on 100/120 Supelcoport, 1.2 m × 4 mm i.d.; Supelco, Inc., Bellefonte, Pa.
[13] Finnigan 4023 GC/MS; Finnigan MAT, San Jose, Calif.
[14] 3% SP-2100 on 100/120 Supelcoport, 0.9 m × 2 mm i.d.; Supelco, Inc.
[15] Hypersil ODS, 5-μm particle size, 250 × 4.6 mm i.d.; Shanden Southern Instruments, Inc., Sewickly, Pa.
[16] WISP 710; Waters Associates, Inc., Milford, Mass.
[17] Beckman 110A; Beckman Instruments, Inc., Fullerton, Calif.
[18] Model 440; Waters Associates.
[19] Aquasol; New England Nuclear, Boston, Mass.
[20] ACS; Amersham Corp., Arlington Heights, Ill.
[21] Concifluor; Mallinckrodt Chemical Works, St. Louis, Mo.
[22] Spex Freezer/Mill, No. 6700; Spex Industries, Inc., Metuchen, N.Y.
[23] Mark III, 6880; Tracor Analytic Inc., Elk Grove Village, Ill.

samples (150 mg) were processed similarly without prior homogenization. The label activity from feces and skin was then counted with a liquid scintillation counter.

Pharmacokinetic Analysis—Since the choice of a definitive model was considered premature, model-independent techniques were used to describe the pharmacokinetic behavior of probucol. Over the sampling interval the area under the concentration-time curve (AUC) was determined by the trapezoidal rule (5). The first moment of the plasma concentration-time profile (AUMC) was determined similarly, after multiplying each concentration by its time (6). The AUC beyond the last plasma concentration value (AUC_t^∞) and the AUMC beyond that point ($AUMC_t^\infty$) were calculated by Eqs. 1 and 2, respectively. The terminal slope (β), the last plasma concentration (C_{pt}), and the time (t) when C_{pt} occurred were used in that calculation:

$$AUC_t^\infty = \frac{C_{pt}}{\beta} \quad \text{(Eq. 1)}$$

$$AUMC_t^\infty = \frac{C_{pt} \cdot t}{\beta} + \frac{C_{pt}}{\beta} \quad \text{(Eq. 2)}$$

The apparent oral (aCL_o) or systemic (CL_s) plasma clearance, the mean residence time of the drug in the plasma (MRT), and the apparent steady-state volume of distribution (Vd_{ss}) were calculated *via* Eqs. 3–6. After oral administration:

$$aCL_o = \frac{F \cdot \text{Dose}}{\text{AUC}} \quad \text{(Eq. 3)}$$

where F was the fraction of the dose absorbed and:

$$\text{MRT} = \frac{\text{AUMC}}{\text{AUC}} - \frac{1}{k_a}, \quad \text{(Eq. 4)}$$

where k_a was the absorption rate constant. After intravenous administration:

$$CL_s = \frac{\text{Dose}}{\text{AUC}} \quad \text{(Eq. 5)}$$

$$\text{MRT}_{iv} = \frac{\text{AUMC}}{\text{AUC}} \quad \text{(Eq. 6)}$$

The apparent steady-state volume of distribution (Vd_{ss}) was the product of CL_s and MRT_{iv}. The absorption was presumed to be a first-order rate phenomenon. An estimate of the absorption rate constant (k_a) was determined from a nonlinear least-squares parameter optimization computer program (NONLIN) (7) that assumed the plasma concentration-time profile of oral drug could be described by a first-order rate of absorption and biphasic first-order rates of elimination, such that $C_{p(oral)} = Ae^{-\alpha t} + Be^{-\beta t} + Me^{-k_a t}$. The half-life was calculated by dividing the ln 2 by the terminal slope (β).

A prediction of the mean plasma concentration at steady-state ($\overline{C}_p^{ss}$) was obtained from Eq. 7 which employs the accumulation ratio (8), MRT/τ, the dosage interval (τ), and the mean plasma concentration, $\overline{C}_p = (\text{AUC}/\text{MRT})$, after a single oral dose:

$$\overline{C}_p^{ss} = \frac{\text{MRT}}{\tau} \cdot \overline{C}_p \quad \text{(Eq. 7)}$$

RESULTS

The Pharmacokinetics of Probucol in Blood Plasma—After the 10-mg/kg intravenous dose, plasma probucol concentrations decreased over the first 20-min period and then increased fourfold such that concentrations of probucol over the next 12 h were greater than concentrations observed just after administration. In a separate group of rats the concentrations of label activity in plasma (expressed as microgram equivalents of probucol per milliliter) mimicked the decrease and subsequent increase in probucol concentrations (Fig. 1A). Peak concentrations of probucol were observed 8–12 h after the intravenous dose and a biexponential first-order rate of elimination of probucol from plasma then occurred. The half-life of the terminal elimination phase was 6.1 d, and the apparent systemic clearance of probucol was 21 mL/h/kg (Table I). Only 11–23% of the label concentration in plasma after the labeled intravenous dose was attributed to labeled metabolites of probucol.

After the 10-mg/kg oral dose of probucol, peak plasma concentrations of drug occurred within 2 h of administration (Fig. 1A). Initially, during the absorption phase, concentrations of probucol were greater than concentrations of label activity. This phenomenon was attributed to the fact that a different

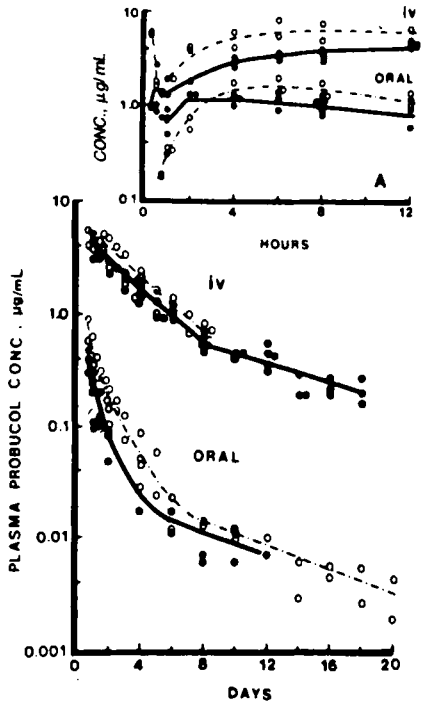

Figure 1—*Plasma concentrations of probucol (●) and [^{14}C]probucol equivalent(s) (○) after a single 10-mg/kg oil–water emulsion dose of probucol to male Sprague–Dawley rats. Inset A shows the initial 12 h of the graph in greater detail.*

group of rats was used to define the label and probucol plasma curves. The elimination of probucol from plasma consisted of two phases, and the half-life of the terminal phase was ~5.6 d. The mean residence time (MRT) of probucol was 2.36 d and the apparent oral plasma clearance was 25.0 mL/h/kg (Table I). The plasma concentration-time curve of probucol and the curve suggested by the corresponding plasma concentrations of label activity were parallel. The difference between the AUC values of the two profiles during the first 8 d indicated that 25–48% of the label activity in plasma was associated with metabolites of probucol.

Tissue Distribution—One hour after intravenous administration, 72% of the dose was found as probucol in the liver; the concentration in liver was 130–200 times the concentration in plasma (Fig. 2). After 7 d, the liver still contained more of the radioactivity (8.2%) than any other tissue studied (Table II). On day 8, 5.6% of the dose was found in the liver as unchanged drug. The half-life during the terminal phase of probucol elimination from liver was 5.5 d.

In contrast to the distribution of intravenous probucol, 7 d after the 10-mg/kg oral dose of [^{14}C]probucol, the highest concentrations of radioactivity were found in the adrenals and in brown and body fat (Table II). Concentrations in the liver after 7 d were <10% of those observed in brown fat. The largest fraction of the oral labeled dose was detected in muscle and body fat when the study was terminated (day 7). Based on reported values for body fat and muscle mass for the rat (9), these two tissues on day 7 contained 0.44 and 0.58% of the labeled dose, respectively.

Excretion—After intravenous administration, only 19% of the labeled dose was excreted in feces within the first 48 h, but after oral administration, 92% of the labeled dose was detected in feces within 48 h (Table III). By the seventh day, 94% of the labeled oral dose was recovered in the feces, in contrast to only 45% of the intravenous dose. Most of the remaining label activity from the intravenous dose was found in tissues (42.5%) (Table II). Less than 5% of the labeled intravenous dose was recovered in urine over the 7-d test period, and negligible amounts of the labeled dose were detected in CO_2.

To determine the degree to which the labeled dose was excreted in the bile, the bile duct was cannulated and animals were given [^{14}C]probucol 3 d after cannulation. After intravenous administration, 5% of the labeled dose was excreted in bile in the first 24 h, a value comparable to the 6% excreted in the feces of noncannulated rats given the intravenous dose (Table III). The rate of elimination of label in bile over the first 24-h interval after intravenous dosing appeared to be a zero-order process since ~1.3% of the labeled dose was excreted in bile during each of the 6-h collection intervals for the first 24 h (Fig. 3). Plasma drug concentrations of the bile duct-cannulated rats 12 and 24 h after intravenous dosing were comparable to the concentration of probucol in noncannulated rats (Table IV).

[24] Biological Materials Oxidizer; R. J. Harvey Instrument Corp., Hillsdale, N.J.

Table I—Parameters Describing the Pharmacokinetic Behavior of Probucol and Label Activity in Plasma of Male Sprague–Dawley Rats [a]

Parameter	Oral		Intravenous	
	Probucol	[^{14}C]Probucol Equivalents	Probucol	[^{14}C]Probucol Equivalents
A, μg/mL	1.82 ± 0.13	—	—	—
α, d^{-1}	2.00 ± 0.10	1.65 ± 0.06	—	—
B, μg/mL	0.0236 0.15	—	—	—
β, d^{-1}	0.122 ± 0.204	0.162 ± 0.107	—	—
k_a, d^{-1}	13.3 ± 1.0	—	—	—
AUC_0^t, μg/mL·d	1.01	1.61	18.2	19.2
where t = d	(12)	(12)	(18)	(8)
AUC_0^∞, μg/mL·d	1.07	1.41	19.9	(26.0)[b]
MRT, d	2.36	2.20	6.7	(6.3)[b]
Terminal $t_{1/2}$, d	5.6	5.9	6.1	(6.1)[b]
F (Fraction absorbed)	0.064	0.064	—	—
aCL_o, mL/h/kg	25.0	—	—	—
CL_s, mL/h/kg	—	—	21.0	—
Vd_{ss}, L/kg	—	—	3.38	—

[a] Given a single oral or intravenous dose of 10 mg/kg of a probucol emulsion. Data expressed as mean ± *SD*. [b] Terminal slope (β) of intravenous probucol was employed to obtain these estimates.

For the first 24 h after oral administration ~0.8% of the labeled dose was recovered in the bile of bile duct-cannulated rats (Table III), and the apparent first-order label elimination half-life during the interval was ~6 h (Fig. 3). Approximately 93.6% of the labeled dose was recovered in the feces of orally dosed noncannulated rats by day 7, and 92% of the dose was fecally excreted in the first 48 h. In the 0–24-h bile collection, only 0.8% of the labeled dose was recovered. During the 24–48-h interval, bile was not collected but must have contained <0.8% recovered in the 0–24-h interval because biliary label excretion declined in a first-order fashion during the 0–24-h interval; only 0.7% of the labeled dose was excreted in the feces during the 48–72-h collection interval (Table III). Thus, the sum of the amount of labeled dose recovered in the 0–168-h urine samples (0.3%), the amount in tissues at 168 h (3.1%), the amount excreted in the 0–24-h bile (0.8%), the amount expected to be excreted in bile during the 24–48-h interval (0–0.7%), and the amount excreted in feces during the subsequent 48–168-h interval (1.5%), provided an approximation of the absorbed labeled dose (5.7–6.4%).

DISCUSSION

Probucol is highly lipophilic (10), and tablet dosage forms are made with micronized drug. Clinical pharmacokinetic studies in healthy volunteers and in hypercholesterolemic patients after single and repeated oral doses of probucol have demonstrated aspects of the steady-state distribution and clearance of probucol (3). Although extensive studies have been conducted in the rat and monkey to elucidate the pharmacodynamic features of probucol, little is known of the bioavailability and pharmacokinetic disposition of probucol and its metabolites in these test species.

In this rat study, ~6% of the administered [^{14}C]probucol was absorbed from the oil–water emulsion. As described earlier, this approximation was the sum of all the label excreted in urine (0–168 h), bile (0–24 h), the biliary excretion estimate (24–48 h), feces (48–168 h), and residual radioactivity in tissues and carcass 7 d after dosage. Another estimate of absorption (5.3%) was obtained from a comparison of the oral and intravenous AUC values, but the apparent nonlinear distribution and/or elimination of probucol over the 0–6-d interval make this estimate less reliable (Fig. 1). Comparisons of the radioactivity in the urine and in various tissues 7 d after the oral and intravenous regimens suggest that the bioavailability of this probucol formulation was poor. A recent study suggests that the drug is selectively absorbed into the lymph rather than the portal circulation and that some oleaginous vehicles enhance its absorption (11).

Although the terminal slopes of the plasma concentration–time curves after the oral and intravenous regimens were similar, a comparison of the preterminal phase of elimination after oral and intravenous administration of probucol suggests that the disposition of the intravenous probucol is nonlinear

Table II—Radioactivity in Tissues and Excreta 7 d after the Administration of [^{14}C]Probucol to Male Sprague–Dawley Rats [a]

	Intravenous Study		Oral Study	
	μg eq/g	Percent of Dose	μg eq/g	Percent of Dose
Tissue				
Adrenal glands	98.1 ± 36.7	0.24 ± 0.052	3.22 ± 1.19	0.010 ± 0.005
Brown fat	29.7 ± 12.8	0.26 ± 0.064	2.19 ± 0.64	0.031 ± 0.011
Liver	19.5 ± 1.6	8.21 ± 0.95	0.17 ± 0.08	0.078 ± 0.037
Body fat[b]	8.4 ± 1.9	4.98 ± 1.37[c]	0.69 ± 0.36	0.44 ± 0.22[c]
Lung	7.3 ± 2.3	0.45 ± 0.15	0.08 ± 0.07	0.004 ± 0.003
Kidney	5.0 ± 0.41	0.38 ± 0.030	0.26 ± 0.10	0.021 ± 0.0064
Thymus	4.6 ± 1.2	0.13 ± 0.045	0.34 ± 0.19	0.008 ± 0.0044
Muscle	2.1 ± 0.35	7.95 ± 1.76[c]	0.13 ± 0.08	0.58 ± 0.36[c]
Skin	5.88 ± 1.67	11.32 ± 2.08	0.511 ± 0.133	1.02 ± 0.13
Tissues + carcass	—	42.43 ± 3.90	—	3.08 ± 0.45
Excreta				
Urine	—	4.65 ± 0.81	—	0.28 ± 0.09
Feces	—	45.26 ± 5.28	—	93.6 ± 1.0
Label recovery	—	92.8 ± 3.9	—	97.1 ± 0.9

[a] A 10-mg/kg single oral or intravenous dose. Data expressed as mean ± *SD* of four rats. [b] Abdominal and perirenal fat. [c] Percent dose calculated, assuming weight of fat is 7.1% or muscle is 45.5% of live body weight (9).

Table III—The Cumulative Percent of the Radioactive Dose Excreted in the Urine and Feces of Male Rats and Biliary Excretion in Bile Duct-Cannulated Male Rats [a]

Day	Intravenous			Oral		
	Urine	Feces	Bile	Urine	Feces	Bile
1	1.06 ± 0.15	6.00 ± 1.12	5.04 ± 0.90	0.095 ± 0.027	—	0.78 ± 0.20
2	2.33 ± 0.41	19.14 ± 2.89	—	0.190 ± 0.070	92.12 ± 1.26	—
3	3.48 ± 1.14	27.32 ± 2.58	—	0.228 ± 0.083	92.82 ± 1.12	—
7	4.65 ± 0.81	45.26 ± 5.28	—	0.28 ± 0.09	93.60 ± 0.96	—

[a] A 10-mg/kg [^{14}C]probucol dose. Data expressed as mean ± *SD*; n = 4 for urine and feces data; n = 5–6 for bile data.

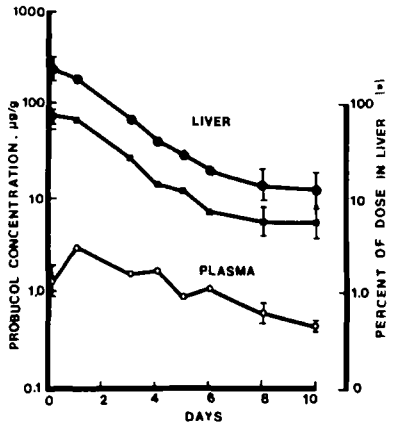

Figure 2—*Concentrations of probucol in plasma (○), liver tissue (●), or percent of dose in liver tissue (■) after a single intravenous dose of 10 mg/kg to male Sprague-Dawley rats.*

(Fig. 1). Also, the unusual decline and recurrence of probucol concentrations seen shortly after intravenous administration, but not after oral administration (Fig. 1A), suggest that the disposition of the intravenous dose may be nonlinear. Differences in the initial (0–24-h) elimination rates of radioactivity in bile and differences in the relative tissue distribution of the oral and intravenous regimens also indicate that the disposition of the intravenous [^{14}C]-probucol dose is concentration dependent.

The apparent zero-order rate of elimination of label activity in bile is seen only after intravenous administration. This may be a consequence of concentration-dependent elimination of the higher concentrations associated with the intravenous dose or the zero-order dissolution of microprecipitated drug. Since oral regimens of 100–1000 mg/kg/d in Sprague-Dawley rats did not elevate serum bilirubin (12), cholestasis is not responsible for this apparent zero-order elimination. Subsequent first-order rates of elimination of probucol and label activity from the plasma and liver tissue indicate that the zero-order rate of elimination is short-lived.

Both oral and intravenous studies indicate that an absorbed dose of probucol is excreted primarily in the feces; the elimination of label in bile duct-cannulated rats (Table III) suggests that most of the label body burden is secreted in the bile. Also, since the amount of radioactivity excreted into bile (0–24 h) is comparable to the amount of labeled dose excreted in feces after intravenous administration (0–24 h), little enterohepatic cycling may occur. The similar plasma concentrations 12 and 24 h after intravenous administration in bile duct-cannulated and noncannulated rats also indicate that enterohepatic cycling would have little effect on circulating plasma probucol concentrations.

The unusual pharmacokinetic behavior of probucol and radioactivity in plasma during the first 12 h after intravenous administration is difficult to explain (Fig. 1A). This effect (the valley in plasma concentration shortly after administration as well as concentrations of the drug from 4 to 12 h that superseded the concentration directly after injection) may be seen with drugs that undergo enterohepatic circulation; but in this study the effect may occur too fast (0–30 min) for such an explanation. Moreover, the radioactivity ex-

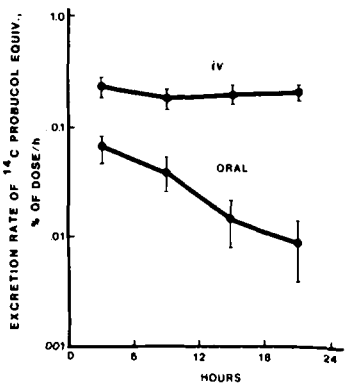

Figure 3—*Biliary excretion rates of [^{14}C]probucol equivalents from rats given a single oral (n = 5) or intravenous (n = 6) 10-mg/kg dose of [^{14}C]-probucol.*

Table IV—Plasma Probucol Concentrations Following Intravenous Administration of Probucol to Rats[a]

Hour	No Cannula	Bile Duct Cannula
12	4.5 ± 0.3 (3)	3.8 ± 1.7 (6)
24	3.5 ± 0.9 (3)	3.7 ± 0.8 (4)

[a] Dose, 10 mg/kg. Expressed as mean (µg/mL) ± *SD*; numbers in parentheses indicate number of animals.

creted in bile is similar to that recovered in feces over a 24-h collection period, and plasma concentrations in bile duct-cannulated rats are comparable to those of noncannulated rats. Consequently, significant recycling is unlikely.

Alternatively, fat and fat-containing emulsions given intravenously tend to accumulate in hepatic tissue (13) and, subsequently, are cleared in a manner comparable to the clearance of chylomicrons. In the present study, the oil from the oil-water emulsion of probucol may accumulate in the hepatic tissue, create a partititoning depot in the liver (since in this study 72% of the intravenous dose was found in the liver at 1 h), and then govern the release of probucol into the systemic circulation. The unusual behavior of the plasma concentration-time curve of carbamazapine, another compound with low aqueous solubility, during intravenous infusion is similar (14). In that study, carbamazapine was solubilized in vehicles containing high concentrations of propylene glycol and given as an infusion. A precipitous fall in plasma concentrations of carbamazapine began shortly after the infusion was initiated. Although Levy *et al.* (14) conducted several experiments to characterize the effect, no definitive explanation was found. Precipitation of the drug in plasma and subsequent redissolution was proposed.

Since the mean residence time of probucol in plasma was 2.4 d after the 10-mg/kg oral dose, with a regimen of 10 mg/kg/d less than a twofold increase in the mean plasma concentration is expected before steady-state $\overline{C}_p^{ss}$ is achieved (nonlinear distribution and/or clearance is not operative in this concentration range). The pharmacokinetic behavior of larger oral doses or an increased number of doses per day, however, may become concentration dependent (indicated by the nonlinear distribution and clearance of the intravenous plasma probucol concentrations) and exhibit another steady-state profile. Dietary and/or formulation factors may also modify the bioavailability, and hence the anticipated effects, of long-term exposure to the drug.

Although the total body clearance is known, the metabolic clearance of probucol is not. Differences between the probucol concentrations and the radiolabeled microgram equivalent concentrations of probucol in plasma indicate the presence of labeled metabolites. Studies of the metabolites of probucol, however, are limited to the characterization of ^{13}C- and ^{14}C-labeled products in the liver and adipose tissue of the rhesus monkey. In both tissues most of the ^{14}C-labeled material was probucol. Six metabolites were also identified in the fat and liver of the monkey[25].

The pharmacokinetic behavior of this formulation of probucol demonstrates not only the poor availability of such a dosage form but also the nonlinear distribution that may be a consequence of the formulation and/or the dose of probucol. This study illustrates the fate of probucol in rats and the potential for accumulation associated with repeated oral regimens of probucol. The need for further investigation of oleaginous vehicles used in preclinical studies is apparent.

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2-Acetylpyridine Thiosemicarbazones XI: 2-(α-Hydroxyacetyl)pyridine Thiosemicarbazones as Antimalarial and Antibacterial Agents

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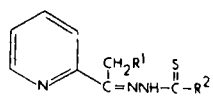
Abstract □ A series of 2-(α-hydroxyacetyl)pyridine thiosemicarbazones was synthesized as potential antimalarial and antibacterial agents. Their synthesis was achieved by the condensation of N^4-mono- or N^4,N^4-disubstituted thiosemicarbazides with 2-(α-hydroxyacetyl)pyridine. The latter was prepared by selective bromine oxidation of (2-pyridinyl)-1,2-ethanediol. The new compounds show potent inhibitory activity against penicillin-sensitive as well as penicillin-resistant *Neisseria gonorrhoeae* (MIC, 0.5–0.004 μg/mL), against *Neisseria meningitidis* (MIC, 0.5–0.032 μg/mL), and *Staphylococcus aureus* (MIC, 0.5–2 μg/mL). Good *in vitro* antimalarial effects against *Plasmodium falciparum* (Smith strain; ID_{50}, 6.7–38 ng/mL) were observed in most of these new agents, but only 3 of 12 compounds exhibit moderate *in vivo* activity against *Plasmodium berghei*. These new agents appear to be less toxic to the host and more water soluble than the corresponding 2-acetylpyridine thiosemicarbazones.

Keyphrases □ Thiosemicarbazone derivatives—2-(α-hydroxyacetyl)pyridine thiosemicarbazones, antimalarial and antibacterial activity □ Antimalarial agents—potential, 2-(α-hydroxyacetyl)pyridine thiosemicarbazones □ Antibacterial agents—potential, 2-(α-hydroxyacetyl)pyridine thiosemicarbazones

Members of a series of N^4-mono- and N^4,N^4-disubstituted 2-acetylpyridine thiosemicarbazones (I) have been reported by us to possess antimalarial (1, 2), antibacterial (3), and antiviral (4) properties. Based on these studies, the structure–activity relationship of this class of compounds has been defined. Thus, N^4,N^4-disubstitution of the thiosemicarbazone moiety appears to be essential for optimal activity against *Plasmodium berghei* in the mouse (2) and several bacterial genera (3). Replacement of the sulfur atom with oxygen leads to inactive compounds. Furthermore, biological activity is limited to those compounds in which the ethylidene group is attached to the 2-position, rather than the 3- or 4-position, of the pyridine ring. The effects resulting from placement of a functional group on the ethylidene function, however, have not been studied. N^4,N^4-Disubstituted 2-acetylpyridine thiosemicarbazones, in particular, exhibit potent antimalarial properties *in vitro* as well as *in vivo*. Against *P. berghei* in mice, the 4-(2-pyridinyl)piperazine and 3-azabicyclo[3.2.2]nonane analogues produced cures of all infected test animals at a dosage of 80 and 160 mg/kg, respectively (2). Although potent inhibitory activity against *Plasmodium falciparum* (Smith) *in vitro* (ID_{50} = 3.6 ng/mL) was observed, 2-acetylpyridine 4,4-dimethyl-3-thiosemicarbazone shows no significant *in vivo* activity against *P. berghei* in mice due to its high host toxicity.

As antibacterial agents, 2-acetylpyridine thiosemicarbazones show excellent activity against both penicillin-sensitive and -resistant strains of *Neisseria gonorrhoeae* with MIC (minimum inhibitory concentration) values in the range of 0.002–0.062 μg/mL. These agents were also found to inhibit the growth of *Neisseria meningitidis* as well as *Staphylococcus aureus*, with the MIC in the range of 0.016–0.062 μg/mL and 0.125–0.5 μg/mL, respectively. 2-Acetylpyridine 4,4-dimethyl-3-thiosemicarbazone [I, $R^2 = N(CH_3)_2$] is by far the most potent compound against *N. gonorrhoeae* among the analogues tested, with an MIC of 0.002–0.008 μg/mL. However, the substantial toxicity observed in mice (2) has limited its potential as a clinical agent.

In the search for agents with lower toxicity, increased water-solubility, and improved structure–activity relationship, the synthesis of a new series of 2-(α-hydroxyacetyl)pyridine thiosemicarbazones (II) was undertaken. These compounds were screened for their antimalarial properties as well as their antibacterial activity.



I, $R^1 = H$
II, $R^1 = OH$

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