The preliminary model for triazinate pharmacokinetics presented here provides a logical basis for further investigation of the mechanism of triazinate transport and activity in different tumor lines.

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Disposition and Absolute Bioavailability of Furosemide in Healthy Males

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Received October 23, 1981, from the **Drug Dynamics Institute, College of Pharmacy, The University of Texas at Austin, Austin, TX 78712*, the [‡]*Hoechst-Roussel Pharmaceuticals Inc., Somerville, NJ 08876*, and the [§]*Warner-Lambert Company, Ann Arbor, MI 48105.* Accepted for publication December 10, 1981.

Abstract □ Furosemide (40 mg) was administered to 18 healthy adult males as an intravenous dose, an oral solution, and in tablet form. The pharmacokinetics of intravenous furosemide were studied, determining a total body clearance rate of 117.6 ± 41.3 ml/min and a harmonic mean half-life of 78 min. The mean absolute bioavailability determined by ratio of areas under the plasma–time curves was 64 and 71% for the solution and tablet, respectively. The mean absolute bioavailability determined by the ratio of urinary cumulative excretion data was 61 and 66% for the solution and tablet, respectively. The absolute bioavailabilities of furosemide determined with plasma and urine data were not significantly different. Thus, urine data alone may be used to establish bioavailability of furosemide. Inspection of plasma–time curves revealed secondary maxima in several subjects, suggesting enterohepatic cycling.

Keyphrases □ Furosemide—disposition and absolute bioavailability in healthy males □ Pharmacokinetics—disposition and absolute bioavailability of furosemide in healthy males □ Bioavailability—disposition, furosemide in healthy males

Furosemide is one of a series of anthranilic acid derivatives which is commonly used as a potent diuretic. Depending on the severity of clinical indication for its use, it is usually administered either orally or intravenously. Therefore, it is of interest to determine the bioavailability of oral preparations with respect to intravenous dosing.

Absolute bioavailability of furosemide has been studied previously by several investigators. Intravenous and oral doses of furosemide were administered previously to four subjects and the absolute bioavailability of tablets was determined to be 65%; the oral aqueous solution was 69% bioavailable (1). [^{35}S]Furosemide was administered orally as an aqueous solution to seven volunteers and intravenously to two different volunteers in another study (2). Comparison of the areas under the plasma curves across subjects determined the solution was 67% bioavailable. In a study with six volunteers (3), absolute bioavailability of oral furosemide (dosage form not identified) was found to be 49%. Eleven normal volunteers were studied (4); tablet and solution preparations were determined to be 69% bioavailable.

The present study was conducted to determine the absolute bioavailability of furosemide (40 mg) given in tablet form and as an oral solution to a large population of healthy males. In addition, the feasibility of using urinary excretion data alone to establish bioavailability was investigated. This would allow future bioavailability studies to be conducted without exposing subjects to numerous blood collections.

Absolute bioavailability of furosemide tablets and solution was established by both ratio of the areas under the plasma–time curves and ratio of cumulative excretion data. The disposition of furosemide given intravenously was also determined. Analysis of the resulting data strongly suggests enterohepatic cycling of furosemide.

EXPERIMENTAL

Subject Selection—Twenty-one healthy males, 20–31 years of age (mean 24) weighing between 61 and 83 kg (mean 71), who were in good physical condition as determined by physical examination, volunteered to participate in the study. Informed consent was obtained from each subject[1].

Study Design—An open Latin-square design was used to study 21 subjects divided into three groups of seven. Subjects were randomly as-

[1] The protocol has approval of the University of Texas at Austin Human Investigation Review Committee.

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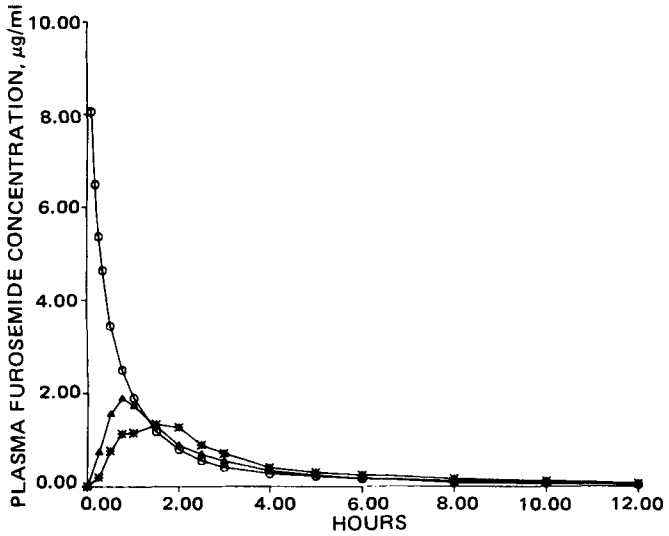

Figure 1—*Mean furosemide plasma concentrations following 40 mg administered as an intravenous solution (○), oral solution (△), and tablet (*) to 18 healthy adult males.*

signed to each group. A single dose of furosemide[2] (40 mg) was administered on 3 different days as an intravenous injection, oral aqueous solution, and tablet. Seven-day washout periods separated the study days.

All subjects abstained from medications, smoking, and alcohol for 1 week prior to and throughout the study. Subjects fasted for 12 hr before each drug administration and 3 hr thereafter. Furosemide tablets were taken with 200 ml of water. Four milliliters of furosemide oral solution was administered with 200 ml of water. The intravenous furosemide dose was injected over a 2-min period.

Following drug administration, blood samples (10 ml) were collected from a forearm vein using a plastic syringe with immediate transfer to heparinized tubes. Blood was collected immediately before and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, and 12 hr after oral drug administration. Blood was collected immediately before and at 5, 10, 15, 20, 30, and 45 min and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, and 12 hr after the intravenous injection was completed. Plasma was separated and frozen at −20° until assayed. During each study day, urine was collected immediately before drug administration and for the following periods: 0–1, 1–2, 2–3, 3–4, 4–5, 5–6, 6–8, 8–12, and 12–24 hr. Urine volumes were recorded and an aliquot was frozen at −20° until assayed.

Assay—The plasma and urine specimens were assayed by reversed-phase high-performance liquid chromatography (HPLC) using a method described previously (5). The only substantive change in procedure was the use of hydroflumethiazide as internal standard instead of furosemide methyl ester. The methods described in the literature and used in the author's laboratories are specific for the intact furosemide and showed no interference from its hydrolysis product, 4-chloro-5-sulfamoyl anthranilic acid. Furthermore, no interference was observed at the retention time for the internal standard, hydroflumethiazide.

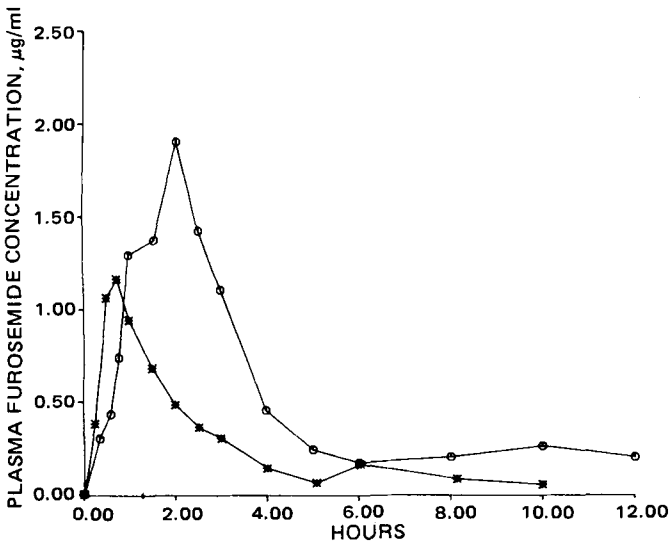

Figure 2—*Furosemide plasma concentrations following 40 mg administered as an intravenous solution to two healthy adult males (Subjects 2 and 13).*

[2] Lasix, supplied by Hoechst-Roussel Pharmaceuticals Inc., tablet: Lot #601160; oral solution: Lot #680010; intravenous solution: Lot #619513.

Table I—Pharmacokinetic Parameters (Mean ± *SD*) in 16 Normal Males Following Intravenous Administration of Furosemide (40 mg)

Parameter	Value
A, μg/ml	8.7 ± 2.7
B, μg/ml	2.1 ± 1.3
α, min^{-1}	0.063 ± 0.051
β, min^{-1}	0.0089 ± 0.0052
Ke, min^{-1}	0.028 ± 0.014
K_{21}, min^{-1}	0.021 ± 0.017
K_{12}, min^{-1}	0.024 ± 0.027
$AUC_{0\to\infty}$ μg $min\ ml^{-1}$	374.28 ± 110.49
Vc, liter/kg	0.056 ± 0.017
Vd_{ss}, liter/kg	0.13 ± 0.06
Cl_T, ml/min	117.6 ± 41.3
Cl_R, ml/min	88.9 ± 44.8 (n = 13)
Cl_{NR}, ml/min	32.4 ± 11.9 (n = 13)

The analytical method employed involved extraction of acidified urine or plasma (spiked with internal standard) with a fivefold excess of diethyl ether. In the case of plasma, the ether was reduced to dryness under a stream of nitrogen, the residue reconstituted in glycine buffer (pH 11.0), and subjected to chromatographic development. Urine ether extracts were back-extracted with glycine buffer (pH 11.0) and the latter alkaline extracts subjected to HPLC. Peak areas (furosemide and internal standard) were determined with a computing integrator dedicated to the HPLC apparatus used in the determinations. Peak area ratios from samples were compared with similar data developed from eight standard samples for plasma determinations (in the range of 0.05–20.05 μg/ml) and five standard samples for urine determinations (in the range of 0.1–50.0 μg/ml), which were used in preparing standard curves ($r \geq 0.999$). The average relative standard deviation of the standard samples for plasma determinations was 7.3%; 2.6% was found for urine determinations. All samples were protected from light prior to analysis.

Data Analysis—Area under the plasma concentration–time curve (AUC) was calculated for 0–12 hr using the trapezoidal rule. The $AUC_{0\to\infty}$ for intravenously administered furosemide included a terminal slope correction factor, Cp^n/β, where Cp^n is the last measured concentration–time point, and β is the slope of the terminal log-linear phase of the semilog plot of concentration *versus* time. The maximum plasma concentration achieved (Cp_{max}) and time to maximum plasma concentration (t_{max}) were observed from the measured plasma concentrations following oral drug administration.

Plasma concentration data following termination of the rapid intravenous infusion were fitted to the biexponential equation:

$$Cp = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{(Eq. 1)}$$

where Cp is the plasma concentration in micrograms per milliliter at time t, A and B are pre-exponential terms in units of concentration, and α and β are hybrid first-order rate constants with units of reciprocal time. The data were fitted using nonlinear least-squares regression analysis with the program NONLIN (6) (weight = $1/Cp$). Microconstants were calculated for each individual subject using:

$$K_{21} = \frac{A\beta + B\alpha}{A + B} \qquad Ke = \frac{\alpha\beta}{K_{21}} \qquad K_{12} = \alpha + \beta - K_{21} - Ke$$

(Eq. 2)

The volume of distribution of the central compartment (Vc) was calculated from:

$$Vc = \frac{\text{Dose}}{A + B} \qquad \text{(Eq. 3)}$$

The steady-state volume of distribution (Vd_{ss}) was calculated from the definition:

$$Vd_{ss} = \left(1 + \frac{K_{12}}{K_{21}}\right) Vc \qquad \text{(Eq. 4)}$$

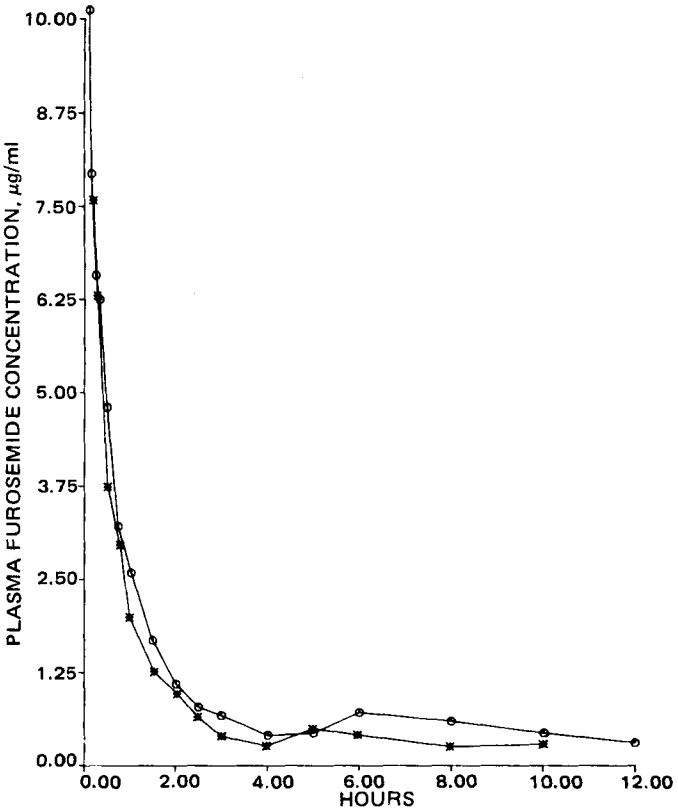

Figure 3—*Furosemide plasma concentrations following 40 mg administered as tablet (*) and oral solution (O) to two healthy adult males (Subjects 8 and 16).*

Total body clearance (Cl_T) was determined from:

$$Cl_T = \frac{\text{Dose}}{AUC_{0\to\infty}} \qquad \text{(Eq. 5)}$$

Renal clearance (Cl_R) was calculated from the relation:

$$Cl_R = \frac{X_{u0\text{-}24}}{AUC_{0\to\infty}} \qquad \text{(Eq. 6)}$$

where $X_{u0\text{-}24}$ is the total amount of unchanged drug eliminated in the urine in 24 hr. The difference between total body clearance and renal clearance was labeled the nonrenal clearance (Cl_{NR}).

The absolute bioavailability (F) was determined from plasma and urine data from:

$$F_{\text{plasma}} = \frac{AUC_{0\text{-}12}^{\text{oral}}}{AUC_{0\text{-}12}^{\text{iv}}} \qquad \text{(Eq. 7)}$$

$$F_{\text{urine}} = \frac{X_{u0\text{-}24}^{\text{oral}}}{X_{u0\text{-}24}^{\text{iv}}} \qquad \text{(Eq. 8)}$$

Analysis of variance with the least significant difference test utilized for *a posteriori* comparison and Student's t test for paired data were used to make statistical evaluations of the data. An α-level of <0.05 was accepted as evidence of statistical significance.

RESULTS AND DISCUSSION

Eighteen subjects completed the study. Three subjects dropped from the study for reasons unrelated to the administered drug. Data collected from these three subjects were not included in the data analysis.

Mean plasma furosemide concentrations given intravenously and orally are depicted in Fig. 1. Two subjects administered the intravenous dose had a second peak in their plasma furosemide concentrations at 5–6 hr. The data from these two subjects were not included in the determination of the disposition of intravenous furosemide. The pharmacokinetic parameters for the remaining 16 subjects are given in Table I. The mean terminal log-linear phase disposition rate constant corresponds to a half-life of 78 min. This is reasonably consistent with other half-life values of 26–72 min following intravenous furosemide dosing (7). Other pharmacokinetic parameters are in agreement with those previously reported in the literature, though the total body clearance of 118 ml/min is among the lower clearance values reported (112–268 ml/min) (7).

Table II—Bioavailability Parameters (Mean ± *SD*) in 18 Normal Males Following Administration of Furosemide (40 mg) [a]

Parameter	Value
$AUC_{0\text{-}12}$ iv, μg min ml^{-1}	383.9 ± 121.9
$AUC_{0\text{-}12}$ solution (μg min ml^{-1})	240.9 ± 98.8
$AUC_{0\text{-}12}$ tablet (μg min ml^{-1})	250.0 ± 116.2
$X_u^{0\text{-}24}$/dose iv	0.71 ± 0.10 (n = 14)
$X_u^{0\text{-}24}$/dose solution	0.41 ± 0.02 (n = 16)
$X_u^{0\text{-}24}$/dose tablet	0.44 ± 0.15
$AUC^{\text{solution}}/AUC^{\text{iv}}$	0.64 ± 0.22
$AUC^{\text{tablet}}/AUC^{\text{iv}}$	0.71 ± 0.35
$X_u^{\text{solution}}/X_u^{\text{iv}}$	0.61 ± 0.17 (n = 12)
$X_u^{\text{tablet}}/X_u^{\text{iv}}$	0.66 ± 0.23 (n = 14)
$Cp_{\max}$ solution, μg/ml	1.8 ± 0.6
$Cp_{\max}$ tablet, μg/ml	1.7 ± 0.9
$t_{\max}$ solution, min	50.1 ± 23.8
$t_{\max}$ tablet, min	86.2 ± 50.3

[a] Given as tablet, oral solution, and intravenous solution.

Individual plasma concentrations in the two subjects with a second peak in plasma furosemide concentrations are displayed in Fig. 2. The appearance of this second peak suggests the possibility of biliary secretion of furosemide and/or metabolite(s) with subsequent reabsorption of the parent drug. An attempt to study biliary secretion of furosemide in humans was made previously (2). This group found increased radioactivity counts in duodenal aspirates of two subjects given intravenous [^{35}S]furosemide with cholecystokinin stimulation. It was not determined if the increased counts represented intact furosemide or metabolites. [^{35}S]-Furosemide was administered intravenously to two dogs, and an average of 51.4% was found in the feces (8). It was deduced that biliary secretion may play a major role in the elimination of furosemide and/or metabolites. In additional work, cannulation of the bile duct with complete bile collection in one dog given intravenous furosemide showed furosemide to be present in the bile[3]. Secondary maxima have been reported in normal subjects given oral furosemide, and biliary recycling was suggested as an explanation (9).

While no conclusive studies have been conducted in humans to determine if furosemide undergoes enterohepatic cycling, the presence of intact furosemide in the bile of one animal species and the observed secondary maxima in the plasma concentration–time curves in two subjects in the present study suggest enterohepatic cycling may occur. This hypothesis is further supported by plasma concentration–time curves obtained from data following oral doses of furosemide. Secondary maxima were seen in several subjects receiving the drug orally; representative curves are shown in Fig. 3.

Mean bioavailability parameters are displayed in Table II. The AUC was truncated at 12 hr because secondary maxima in plasma concentrations obscured the terminal log-linear phase in many subjects, making corrections to $AUC_{0\to\infty}$ unreliable. Because most subjects had plasma concentrations below assay sensitivity or near the sensitivity limit at the time of the last blood sample, a significant portion of the AUC was not lost by not calculating the AUC to infinity. The $AUC_{0\text{-}12}$ for the intravenous dose was significantly greater than the $AUC_{0\text{-}12}$ for either the oral solution or tablet. The mean absolute bioavailability determined from the plasma data was 64 and 71% for the solution and tablet, respectively. The bioavailabilities for the solution and tablet preparations were not statistically significantly different for either the plasma or urine determinations.

The absolute bioavailabilities of furosemide determined with plasma and urine data were not significantly different ($p > 0.05$); however, there was a trend toward a greater bioavailability ratio determined from plasma data, compared with the ratio determined from the urine data. This is similar to previously reported data (2), in which 67% bioavailability of furosemide solution determined from plasma data and 65% bioavailability determined from urinary data was reported. In contrast, 49% bioavailability determined from plasma data was reported and 52% bioavailability determined from urinary data (3). The slightly greater bioavailability determined with plasma data in the present study may possibly be explained by biliary recycling of furosemide. The observed secondary maxima increased the AUC. There was also considerable intrasubject variability in the appearance of these peaks. The bioavailability determined by AUC may be inflated from secondary plasma peaks while the

[3] G. Yakatan and J. Johnston, unpublished data.

bioavailability determined from urine data may give a more accurate reflection of the true bioavailability. Thus, the feasibility of using urinary excretion data alone to determine bioavailability of furosemide is good, given the statistical agreement of absolute bioavailability determined by plasma and urine data. Also, the possibility of overestimation of bioavailability by plasma data because of enterohepatic recycling makes bioavailability determined from urine data appear more reliable.

The t_{max} and Cp_{max} determinations were not statistically significantly different for the tablet and solution. There was a trend toward later peak plasma concentrations following tablet administration, probably due to time required for tablet disintegration and dissolution. The seeming disagreement of Cp_{max} determinations presented in Table II and Fig. 1 is a function of mean data being graphically presented, while the means of individual subjects are presented in the table.

The bioavailabilities of the tablet and solution are essentially the same, though <70% of the dose was absorbed. This suggests the absorption may not be solely dependent on solubility, but may also be limited by absorption occurring only from a specific site in the GI tract. Site-limited absorption may explain intrasubject variability in absolute bioavailability.

In summary, the disposition of intravenous furosemide as determined by this study is in agreement with previous reports. The mean absolute bioavailability determined from cumulative urinary excretion data was 61 and 66% for the solution and tablet, respectively. The bioavailability determined with urine data may be more reliable than bioavailability determined with plasma data because of a possible enterohepatic recycling process. Site-limited absorption of furosemide is suggested.

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Determination of Amine Ingredients in Cough–Cold Liquids by Reversed-Phase Ion-Pair High-Performance Liquid Chromatography

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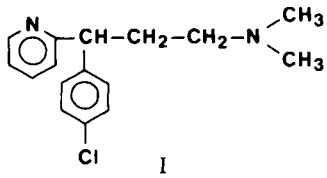
Received October 9, 1981, from *Control Analytical Research and Development, The Upjohn Company, Kalamazoo, MI 49001.* Accepted for publication December 14, 1981.

Abstract □ The chromatographic behavior of phenylephrine, codeine, pseudoephedrine, phenylpropanolamine, methoxyphenamine, pheniramine, pyrilamine, dextromethorphan, and chlorpheniramine was examined by reversed-phase, ion-pair high-performance liquid chromatography. An isocratic chromatographic system was devised for the analysis of cough–cold liquids containing these amine drugs by optimization of the mobile phase ionic strength, buffer pH, pairing ion concentration, and secondary ion concentration. Quantitative recovery and excellent precision were demonstrated for the simultaneous determination of phenylpropanolamine, dextromethorphan, and chlorpheniramine in a typical formulation. The method was successfully applied to various commercial cough–cold liquids for the analysis of a wide range of amine drugs.

Keyphrases □ Cough–cold liquids—determination of amine ingredients by reversed-phase, ion-pair high-performance liquid chromatography □ High-performance liquid chromatography—determination of amine ingredients in cough–cold liquids □ Amine drugs—determination in cough–cold liquids by reversed-phase ion-pair high-performance liquid chromatography

Cough–cold liquids are usually complex formulations containing several active ingredients and a broad spectrum of excipients such as dyes, flavors, sweeteners, and preservatives. Many of these products are designed to be multisymptom preparations typically containing a variety of basic amino compounds acting as antihistamines, decongestants, or cough suppressants. Some of the common amino agents utilized include phenylephrine, phenylpropanolamine, pseudoephedrine, pyrilamine, pheniramine, chlorpheniramine, codeine, and dextromethorphan. The analgesics, phenacetin and acetaminophen, are also commonly found in cough–cold liquids adding further complexity to the list of possible ingredients. Preservatives such as methyl- and propylparaben or sodium benzoate are normally present in a formulation. It was the purpose of this study to develop a simple high-performance liquid



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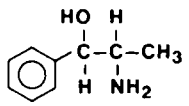

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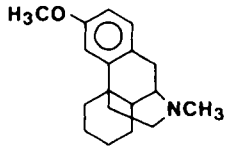

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