Pharmacokinetics of Bumetanide Following Intravenous, Intramuscular, and Oral Administrations to Normal Subjects

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Abstract □ The pharmacokinetics of bumetanide was studied in 12 normal subjects after 1-mg intravenous, intramuscular, oral solution, and tablet administrations in a random four-treatment crossover design. Plasma and urine concentrations of intact bumetanide were analyzed by a sensitive and specific RIA. The pharmacokinetics of bumetanide after intravenous administration was characterized by a biexponential equation, including an initial disposition phase ($t_{1/2,\alpha}$ = 5.1 min), followed by a slower elimination phase ($t_{1/2,\beta}$ = 44 min). Bumetanide pharmacokinetics after intramuscular and oral administration could be described by a biexponential equation with first-order absorption and elimination. Bumetanide is rapidly absorbed *via* the intramuscular and oral routes, with mean ± *SD* maximum plasma concentrations of 38.2 ± 9.8 (intramuscular), 34.0 ± 10.6 (oral solution), and 30.9 ± 14.6 ng/mL (tablet) achieved within 0.34 ± 0.23, 0.76 ± 0.27, and 1.8 ± 1.2 h after dosing, respectively. The drug is rapidly eliminated from the body after intravenous, intramuscular, oral solution, and oral tablet administrations, with half-lives ranging from 24–86, 47–139, 27–71, and 26–99 min, respectively. Approximately 70% of a parenteral dose and 60% of an oral dose are excreted as intact drug in urine taken 0–24 h after administration. The extent of bioavailability of bumetanide from the tablet and oral solution dosage forms are equivalent, and the absolute bioavailability of the intramuscular and oral preparations are ~100 and 80%, respectively. This is consistent with the predicted limited extent of first-pass metabolism after complete absorption of an oral dose. The pharmacokinetic and pharmacodynamic data support the conclusion that bumetanide is galenically available from the tablet dosage form and that comparable cumulative diuretic activity is obtained, regardless of the route and mode of administration.

Keyphrases □ Pharmacokinetics—bumetanide, intravenous, intramuscular, and oral administration □ Bumetanide—pharmacokinetics, intravenous, intramuscular, and oral administration, □ Diuretics—bumetanide, pharmacokinetics following intravenous, intramuscular, and oral administration □ Bioavailability—bumetanide, intravenous, intramuscular, and oral administration

Bumetanide, 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoic acid, a new potent sulfonamide diuretic, produces a rapid diuretic response with a short duration of action in humans. The major site of action is on the ascending limb of Henle's loop, in addition to further action on the proximal tubule (1–3). The diuretic effects of bumetanide are as extensive as those of other "loop" diuretics but at oral doses of 1 mg, as compared with 40–60 mg of furosemide (4–8). Although pharmacokinetic studies in humans after intravenous and oral administration of bumetanide have been reported (6, 9–12), the pharmacokinetics and bioavailability of intramuscularly administered bumetanide have not.

The purpose of this study was to determine primary pharmacokinetic parameters of bumetanide following intravenous, intramuscular, and oral administrations and to assess the bioavailability of the intramuscular, oral tablet, and oral solution preparations of bumetanide relative to intravenous administration.

EXPERIMENTAL SECTION

Clinical Protocol—Twelve healthy adult male and female volunteers [23–40 years old (mean 30); weight, 54–96 kg (mean 70)] were selected for the study. Subjects were considered normal by medical history, physical examination, and blood and urine laboratory tests. An informed written consent was obtained from each volunteer before the study.

Single 1-mg doses of bumetanide were administered to each subject as intravenous, intramuscular, oral solution, and tablet preparations. Subjects were randomly assigned to the four treatments, and there were at least 2 weeks between dosage administrations. The drug was supplied in 0.25-mg/mL ampules for parenteral and oral solution administrations. The intravenous dose was administered over a 2-min period, and the intramuscular dose was injected into the lateral thigh with an intramuscular administration needle. The oral solution dose was administered in a 4-mL solution given with 250 mL of water, and the oral tablet was given with 250 mL of water.

Subjects fasted for 8 h prior to drug administration and remained fasting until the 4-h blood sample was collected. A hospital-prepared lunch, supper, and a bedtime snack were served. No additional salt or highly salted foods were permitted. Water was permitted *ad libitum*.

Blood samples (10 mL) were drawn through an indwelling cannula or by separate venipuncture into oxalated tubes prior to drug administration and at 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, and 24 h after drug administration. Plasma was separated and stored at −17°C until assayed. All urine voided over 12 h before dose and 0–2, 2–4, 4–6, 6–8, 8–12, and 12–24 h after drug administration was collected, and 50-mL aliquots were stored at −17°C until assayed.

Analytical Procedures—Plasma and urine samples were assayed for intact bumetanide using a slight modification of the sensitive and specific RIA procedure of Dixon *et al.* (13). In the present study, the precipitated antibody-bound fraction was quantitated rather than the unbound fraction, and the data were calculated as outlined by Rodbard (14), with a digital computer used for iterative weighted linear regression analysis of logit B/B_0 *versus* log dose. The RIA had a limit of sensitivity of 3 ng/mL of bumetanide with 0.1 mL of plasma or urine.

Pharmacokinetic Analysis—Plasma concentrations following intravenous administration were curve-fitted as follows (15):

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

and concentrations after intramuscular and oral administration were fitted with the following (15):

$$C_p(t) = A'[e^{-\beta(t-t_0)} - e^{-k_a(t-t_0)}] \quad \text{(Eq. 2)}$$

where $C_p(t)$ is the plasma concentration of bumetanide at time t; A, A', and B are coefficients; α is the initial disposition rate constant; β is the terminal elimination rate constant; k_a is the apparent absorption rate constant; and t_0 is the lag time preceding initiation of absorption. A weighted ($1/C_p$) nonlinear regression program [NONLIN (16)] was used to calculate the best estimates of the pharmacokinetic parameters.

The model-estimated parameters were used to calculate the theoretical area under the plasma concentration–time curve (AUCTh), the apparent volume of the central compartment (V_c), and volume of distribution in the postdistributive phase (Vd_{area}) as follows:

$$\text{AUCTh (intravenous)} = A/\alpha + B/\beta \quad \text{(Eq. 3)}$$

$$\text{AUCTh (intramuscular, oral)} = A'/\beta - A'/k_a \quad \text{(Eq. 4)}$$

$$V_c \text{ (intravenous)} = \text{Dose}/(A + B) \quad \text{(Eq. 5)}$$

$$Vd_{area} \text{ (intravenous)} = \text{Dose}/(\text{AUCTh}\cdot\beta) \quad \text{(Eq. 6)}$$

The maximum plasma concentration (C_{max}) and the time (t_{max}) of its occurrence following intramuscular and oral administrations were read directly from the observed plasma concentration–time data. The area under the plasma concentration–time curve from time zero to infinity (AUCTr) were calculated as follows:

$$\text{AUCTr} = \text{AUC}_{0\to t} + C_p(t)/\beta \quad \text{(Eq. 7)}$$

where $\text{AUC}_{0\to t}$ is the area calculated by trapezoidal summation from time zero to the time, t, of the last measurable plasma concentration, $C_p(t)$. For

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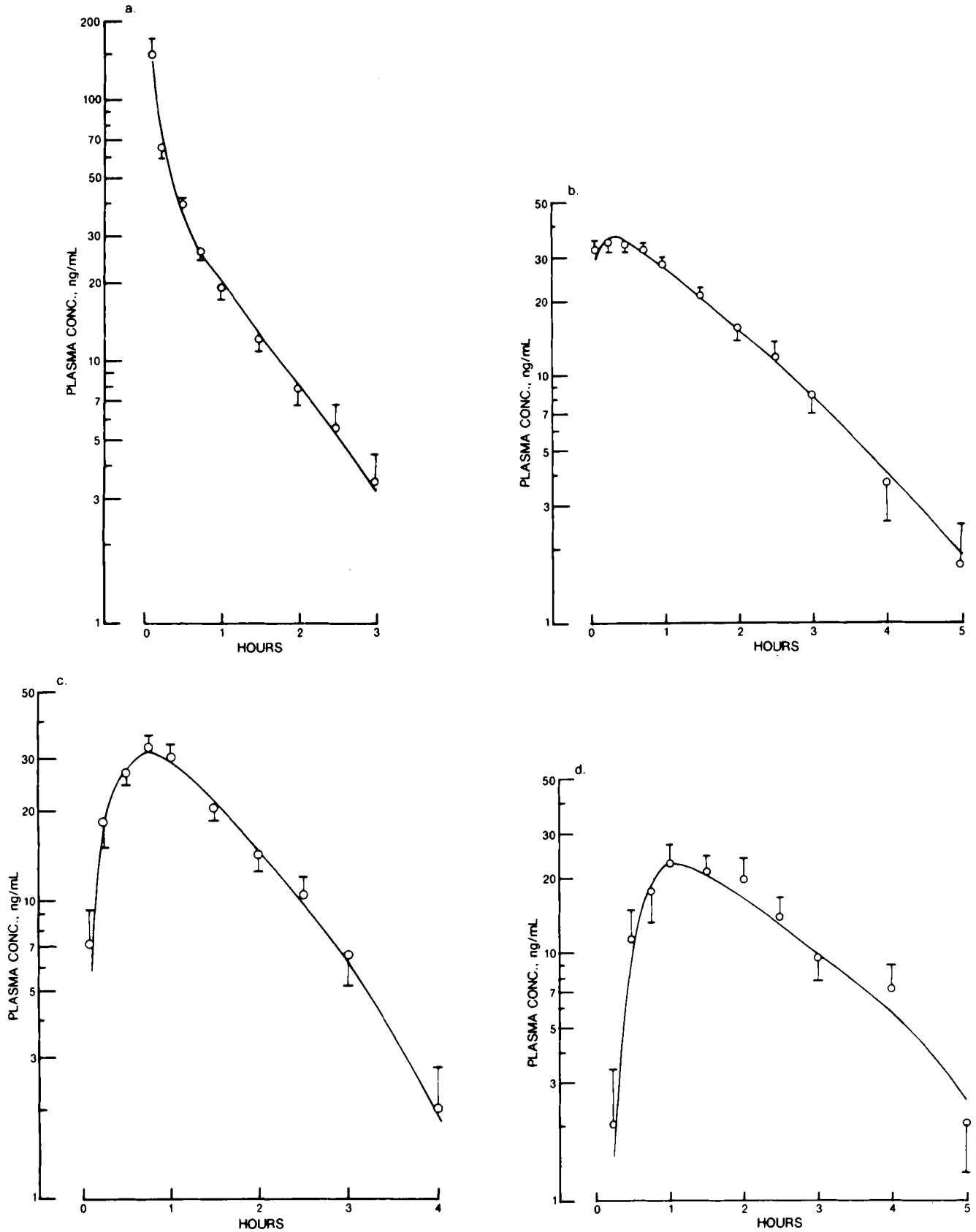

Figure 1—*Mean plasma concentration–time profiles of bumetanide following administrations of 1 mg of bumetanide to 12 normal subjects. The circles are actual data points (mean ±SE), and the solid lines are the fitted curves. Key: (a) intravenous; (b) intramuscular; (c) oral solution; (d) oral tablet.*

intravenous administration, the plasma concentration at time zero was extrapolated graphically.

The volume of distribution at steady-state (Vd_{ss}), systemic plasma clearance (CL_p), apparent oral clearance (CL_o), renal clearance (CL_r), and nonrenal clearance (CL_{nr}) were calculated by model-independent methods from the following relationships:

$$Vd_{ss}\text{ (intravenous)} = \text{Dose (AUMCTr)}/(\text{AUCTr})^2 \quad \text{(Eq. 8)}$$

$$CL_p\text{ (intravenous, intramuscular)} = \text{Dose}/\text{AUCTr} \quad \text{(Eq. 9)}$$

Table I—Pharmacokinetic Parameters[a] of Bumetanide Estimated by Curve-Fitting Intravenous Plasma Concentration–Time Data to Eq. 1

Subject	A, ng/mL	α, h^{-1}	B, ng/mL	β, h^{-1}	$t_{1/2,\alpha}$, min	$t_{1/2,\beta}$, min	V_c, L	Vd_{area}, L	AUCTh, ng·h/mL	r
1	161	6.3	52	0.482	7	86	4.7	15.4	134	0.999
2	117	9.1	74	1.09	5	38	5.2	11.4	81	1.000
3	122	5.7	36	0.797	7	52	6.3	18.9	67	1.000
4	477	7.2	42	0.664	6	62	1.9	11.6	130	0.999
5	329	9.9	32	0.926	4	45	2.8	15.8	68	0.999
6	105	7.0	40	0.854	6	49	6.9	19.0	62	1.000
7	236	6.9	52	0.771	6	54	3.5	12.8	101	0.998
8	157	7.4	46	1.06	5	39	4.9	14.5	65	0.999
9	99	8.5	36	0.909	5	46	7.4	21.6	51	0.999
10	228	8.2	61	0.991	5	42	3.5	11.2	90	1.000
11	81	14.8	84	1.75	3	24	6.1	10.7	54	0.999
12	320	6.7	61	1.01	6	41	2.6	9.1	109	1.000
Mean	203	8.1	51	0.942	5.1[b]	44[b]	4.7	14.3	84	
SD	±120	±2.4	±16	±0.308			±1.8	±3.9	±29	

[a] Parameters are defined in the text. [b] Harmonic mean.

Table II—Model-Independent Pharmacokinetic Parameters Determined from Plasma Concentration–Time Data and Urinary Excretion Data Following 1-mg Doses of Bumetanide[a]

Parameter	Intravenous	Intramuscular	Oral Solution	Oral Tablet
C_{max} (ng/mL)	—	38.2 ± 9.8	34.0 ± 10.6	30.9 ± 14.6
t_{max} (h)	—	0.34 ± 0.23	0.76 ± 0.27	1.8 ± 1.2
AUCTr, (ng·h/mL)	89 ± 30	80 ± 25	64 ± 27	66 ± 28
Vd_{ss} (L)	9.8 ± 3.4	—	—	—
CL_p (mL/min)	208 ± 65	223 ± 59	—	—
CL_o (mL/min)	—	—	299 ± 100	310 ± 165
CL_r (mL/min)	146 ± 59	146 ± 32	169 ± 61	178 ± 99
CL_{nr} (mL/min)	62 ± 24	78 ± 37	130 ± 55	132 ± 71
Percent dose in urine (0–24 h)	69 ± 11	66 ± 9	57 ± 12	58 ± 7
Absolute bioavailability				
Area method	—	0.94 ± 0.25	0.74 ± 0.23	0.78 ± 0.33
Urinary excretion method	—	0.98 ± 0.16	0.84 ± 0.16	0.86 ± 0.13
Relative bioavailability				
Area method	—	—	—	1.0 ± 0.3
Urinary excretion method	—	—	—	1.0 ± 0.2

[a] Parameters are defined in the text. Values are mean ±*SD*; n = 12.

$$CL_o = F\cdot\text{Dose}/\text{AUCTr} \quad \text{(Eq. 10)}$$

$$CL_r = Ae(0\text{–}24\text{ h})/\text{AUCTr} \quad \text{(Eq. 11)}$$

$$CL_{nr} = (CL_p - CL_r) \text{ or } (CL_o - CL_r) \quad \text{(Eq. 12)}$$

where AUMCTr is the area under the first moment of the plasma concentration–time curve, *i.e.*, the area under the curve when the product of time and plasma concentration are plotted against time (17); F is the fraction of the dose that is available for absorption as intact drug (F = 1, assuming complete absorption of the oral dose); and Ae(0–24 h) is the amount of intact bumetanide excreted in the urine over 0–24 h, assuming complete elimination during this interval.

Assessment of the absolute bioavailability of intramuscularly and orally administered bumetanide relative to intravenously administered bumetanide and the relative bioavailability of the oral tablet compared with the oral solution were determined by area ratios and urinary excretion ratios as follows:

Absolute bioavailability

$$= \frac{(\text{AUCTr})\text{ im, oral}}{(\text{AUCTr})\text{ iv}}, \frac{Ae(0\text{–}24\text{ h})\text{ im, oral}}{Ae(0\text{–}24\text{ h})\text{ iv}} \quad \text{(Eq. 13)}$$

Relative bioavailability

$$= \frac{(\text{AUCTr})\text{ tablet}}{(\text{AUCTr})\text{ solution}}, \frac{Ae(0\text{–}24\text{ h})\text{ tablet}}{Ae(0\text{–}24\text{ h})\text{ solution}} \quad \text{(Eq. 14)}$$

RESULTS AND DISCUSSION

Intravenous Administration—The mean observed plasma concentration–time profile and the fitted curve following intravenous administration of bumetanide are shown in Fig. 1a, and the pharmacokinetic parameters estimated by curve-fitting plasma concentration to Eq. 1 are presented in Table I. The mean ±*SD* model-independent pharmacokinetic parameters are presented in Table II. The plasma concentration–time data after intravenous administration were fitted well with a biexponential equation, including disposition and elimination phases in all the 12 subjects. The goodness of fit of the individual data to the equation is shown by correlation coefficients of 0.998–1.000 and the close agreement between the theoretical area [mean ±*SD* = 84 ± 29 ng·h/mL (Table I)] and the model-independent trapezoidal area [mean ± *SD* = 89 ± 30 ng·h/mL (Table II)].

The biphasic plasma concentration–time curve is characterized by a rapid α-phase with a harmonic mean half-life of 5.1 min, followed by a slower β-phase with a harmonic mean half-life of 44 min (Table I; Fig. 1a). Following intravenous administration, bumetanide is rapidly distributed into a small central compartment with a mean volume of 4.7 L ± 1.8 (*SD*) which is roughly comparable to the blood volume in an adult. The mean ±*SD* values for Vd_{area} and Vd_{ss} were 14.3 ± 3.9 and 9.8 ± 3.4 L, respectively. The disposition of intravenously administered [^{14}C]bumetanide in humans has been described by a triexponential equation in an earlier study (11). The mean values reported for $t_{1/2,\alpha}$ (5.9 min), $t_{1/2,\beta}$ (46 min), and V_c (5.5 L) are comparable to the mean values in our present study. However, the reported mean Vd_{area} value of 62.5 L is much higher than the mean Vd_{area} of 14.3 L determined in our study. In the study with labeled drug, the reported Vd_{area} represented the volume of distribution during the terminal phase (γ), which accounted for only 17% of the AUC for bumetanide. In a recent study (12), in which a two-compartment model was shown to adequately fit the data following intravenous administration of bumetanide, the mean values reported for $t_{1/2,\alpha}$ (5.75 min), V_c (3.53 L), and Vd_{ss} (9.45 L) were of the same magnitude as the values obtained in the present study. Their reported mean elimination half-life of 64 min, however, is slightly longer than the mean value of 44 min determined in our study.

Bumetanide is ~96%[1] bound to plasma protein in humans over the therapeutic plasma concentration range. High plasma binding contributes to the relatively small volume of distribution for bumetanide and may contribute to its high renal clearance. Although glomerular filtration is restricted to the free concentration of drug in the blood, active tubular secretion has been shown to act on both free and bound drug concentrations (18, 19). Therefore, binding decreases the volume of distribution of bumetanide, resulting in larger concentrations of drug being delivered to the kidney for possible secretion.

The mean ±*SD* plasma clearance and renal clearance of bumetanide were 208 ± 65 and 146 ± 59 mL/min, respectively (Table II). Lower renal clearance values relative to plasma clearance have been observed in other intra-

[1] Unpublished results; Hoffmann-La Roche, Inc., Nutley, N.J.

Table III—Pharmacokinetic Parameters[a] of Bumetanide Estimated by Curve-Fitting Intramuscular Plasma Concentration–Time Data to Eq. 2

Subject	A', ng/mL	k_a, h^{-1}	β, h^{-1}	t_{lag}, min	$t_{1/2,k_a}$, min	$t_{1/2,\beta}$, min	AUCTh, ng·h/mL	r
1	67	9.6	0.441	0	4	94	146	0.999
2	69	3.1	0.626	0	14	67	88	0.990
3	39	21.1	0.575	0	2	73	65	1.000
4	25	19.2	0.300	0	2	139	80	0.998
5	54	94.9	0.882	0	1	47	61	0.996
6	41	43.8	0.735	0	1	56	55	0.996
7	45	18.8	0.426	0	2	98	103	0.998
8	67	6.1	0.752	0	7	55	78	0.997
9	38	11.9	0.444	4	4	94	82	0.999
10	59	18.1	0.673	0	2	62	84	0.998
11	38	11.5	0.675	0	4	62	53	0.996
12	81	3.2	0.809	0	13	52	75	0.996
Mean	52	21.8	0.612	0	1.9[b]	68[b]	81	
SD	±17	±25.5	±0.177	±0			±25	

[a] Parameters are defined in the text. [b] Harmonic mean.

Table IV—Pharmacokinetic Parameters[a] of Bumetanide Estimated by Curve-Fitting Oral Solution Plasma Concentration–Time Data to Eq. 2

Subject	A', ng/mL	k_a, h^{-1}	β, h^{-1}	t_{lag}, min	$t_{1/2,k_a}$, min	$t_{1/2,\beta}$, min	AUCTh, ng·h/mL	r
1	86	3.8	0.585	4	11	71	124	0.996
2	135	2.3	0.889	3	18	47	93	0.998
3	66	3.1	0.928	3	13	45	50	0.995
4	834	1.7	1.54	4	25	27	53	0.999
5	62	4.1	0.882	2	10	47	55	0.997
6	38	5.1	0.778	4	8	53	41	0.997
7	120	2.5	0.811	0	16	51	100	0.994
8	287	1.7	1.37	13	25	31	40	0.995
9	292	1.9	1.41	8	22	29	56	0.989
10	40	29.5	0.710	26	1	59	55	0.999
11	59	2.6	0.989	12	16	42	37	0.999
12	603	1.0	0.958	1	40	43	50	0.971
Mean	219	4.9	0.988	6.7	8.5[b]	42[b]	63	
SD	±254	±7.8	±0.297	±7.3			±27	

[a] Parameters are defined in the text. [b] Harmonic mean.

venous studies (6, 11, 12) and have been attributed to nonrenal elimination of the drug. The absolute values reported in a recent study (12), however, are much lower than the values in our present study. Evidence that hepatic metabolism and biliary excretion contribute to the total clearance of bumetanide have been reported in previous studies with ^{14}C-labeled bumetanide. After intravenous administration, metabolites of bumetanide accounted for ~30–35% of the total radioactivity excreted into the urine (10, 11), whereas ~14% of an oral dose was recovered in the feces, mostly in the form of metabolites identical to those found in bile (9).

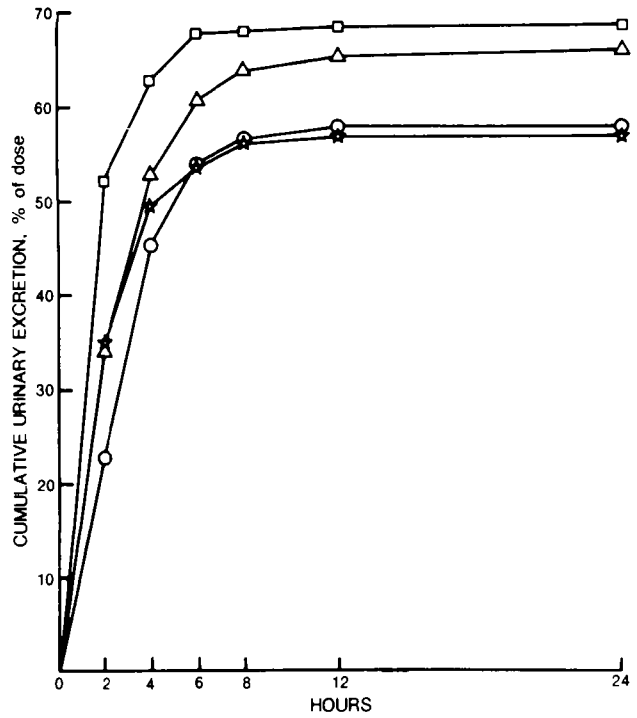

Figure 2—*Mean cumulative urinary excretion of intact bumetanide following administrations of 1 mg of bumetanide to 12 normal subjects. Key: (□) intravenous; (△) intramuscular; (★) oral solution; (○) oral tablet.*

The cumulative urinary excretion of intact bumetanide is shown in Fig. 2. The mean 0–24 h urinary recovery of intact drug following intravenous administration was 69 ± 11%. As shown in Fig. 2, more than one-half of the dose was excreted within 2 h of dosing, and urinary excretion was virtually complete 8 h after drug administration. This rapid excretion of intact drug is consistent with the short duration of action of bumetanide.

Intramuscular Administration—The mean observed plasma concentration–time profile and fitted curve following intramuscular administration are shown in Fig. 1b, and the pharmacokinetic parameters estimated by curve-fitting the plasma concentration–time data to Eq. 2 are presented in Table III. Fits of the intramuscular data to a biexponential equation, including first-order absorption and elimination were good in all 12 subjects, as shown by r values of 0.990–1.000. The goodness of fit of the individual data is supported by the virtually identical mean ±*SD* model-dependent and -independent AUC values (81 ± 25 and 80 ± 25 ng·h/mL, respectively).

Bumetanide was rapidly absorbed following intramuscular administration, with measurable concentrations of intact drug observed in the plasma within 6 min after drug administration. In all but one subject, there was no lag time in the onset of absorption (Table III). The large absorption rate constant (k_a = 21.8 ± 25.5 h^{-1}) further indicates the rapid absorption of bumetanide when administered intramuscularly. After reaching maximum plasma concentrations of 22.5 to 55.4 ng/mL (mean 38.2) within 0.1–0.75 h (mean 0.34) after dosing, plasma concentrations declined rapidly, with a harmonic mean elimination half-life of 68 min. This mean value is longer than the mean of 44 min observed after intravenous administration (Table I). The elimination of bumetanide from the body after intramuscular administration may be absorption rate limited, resulting in a slower apparent elimination rate relative to that after intravenous administration.

The observed mean ±*SD* values for CL_p, CL_r, CL_{nr}, and percentage for the dose recovered as intact drug in the urine were 223 ± 59 mL/min, 146 ± 32 mL/min, 78 ± 37 mL/min, and 66 ± 9%, respectively. These values agree closely with corresponding values observed after intravenous administration (Table II), suggesting that bumetanide is, in fact, completely absorbed into the systemic circulation after intramuscular injection. The absolute bioavailabilities after intramuscular administration were 0.94 ± 0.25 and 0.98

Table V—Pharmacokinetic Parameters[a] of Bumetanide Estimated by Curve-Fitting Oral Tablet Plasma Concentration-Time Data to Eq. 2

Subject	A', ng/mL	k_a, h^{-1}	β, h^{-1}	t_{lag}, min	$t_{1/2,k_a}$, min	$t_{1/2,\beta}$, min	AUCTh, ng·h/mL	r^b
1	72	5.4	0.564	41	8	74	114	0.997
2	77	5.5	0.754	25	8	55	88	0.997
3	40	12.1	0.748	25	8	56	51	0.999
4	389	0.5	0.419	89	83	99	54	0.987 (0.991)
5	128	3.3	1.34	14	13	31	57	0.999
6	64	2.2	0.677	4	19	61	66	0.992
7	637	0.9	0.786	25	46	53	99	0.983 (0.993)
8	262	1.1	0.960	24	38	43	41	0.996
9	241	1.0	0.750	35	42	55	72	0.988
10	261	0.5	0.478	48	83	87	65	0.987 (0.997)
11	443	1.8	1.60	38	23	26	23	0.998
12	40	4.9	0.723	28	9	58	47	0.999
Mean	221	3.3	0.817	33	12.6[c]	51[c]	65	
SD	±190	±3.4	±0.342	±21			±26	

[a] Parameters are defined in the text. [b] Values in parentheses are for zero-order fit. [c] Harmonic mean.

± 0.16 (based on the trapezoidal AUC and the 0–24-h urinary excretion, respectively), indicating that intramuscularly administered bumetanide is completely available relative to intravenously administered bumetanide.

Oral Administration—The mean observed and fitted plasma concentration-time curves following administration of the oral solution and the oral tablet are shown in Fig. 1c and d, respectively. The respective pharmacokinetic parameters estimated by curve-fitting the plasma concentration-time data to Eq. 2 are presented in Tables IV and V. The plasma concentration-time data after administration of both oral formulations were fitted with a biexponential equation, including first-order absorption and elimination. Good fits were obtained in all 12 subjects for the oral solution and in 9 subjects for the tablet. Although the oral tablet data from the other three subjects were also fitted with Eq. 2, there was a slight improvement in the fits when the plasma concentration-time data were fitted with a monoexponential equation with zero-order absorption (Table V). The goodness of fit of the data to the equation selected is shown by r values ranging from 0.971 to 0.999 and 0.983 to 0.999 for the oral solution and tablet, respectively. The good agreement of the model-independent AUC values (Table II) and the AUC values estimated from the curve-fitted parameters (Tables IV and V) further supports the reliability of the equation to fit the data.

After lag times of 0–26 and 4–89 min following administration of the oral solution and tablet, respectively, the harmonic mean half-lives of absorption ($t_{1/2,k_a}$) were 8.5 and 12.6 min, respectively. Mean ±*SD* C_{max} and t_{max} values for the oral solution were 34.0 ± 10.6 ng/mL and 0.76 ± 0.27 h, respectively, whereas the corresponding values for the tablet were 30.9 ± 14.6 ng/mL and 1.8 ± 1.2 h. The mean C_{max} and t_{max} values observed for the tablet are in good agreement with the values for C_{max} of 30 ng/mL and t_{max} of 1.5 h observed in a previous study following a 1-mg oral dose of bumetanide (6). Comparable mean t_{max} values (1.4 h) but higher mean C_{max} values (48 ng/mL) have been reported for a 1-mg oral dose of bumetanide in another study (12). Although absorption from the GI tract was rapid for both formulations, the later mean peak time observed for the tablet reflects the longer lag time, as well as a somewhat slower rate of absorption from the tablet relative to the oral solution. The plasma concentrations declined rapidly after reaching maximum concentrations, with harmonic mean elimination half-lives of 42 min for the oral solution and 51 min for the tablet. The mean half-lives observed after oral administration are comparable to the mean half-life observed after intravenous administration, indicating that the apparent elimination rate of bumetanide from the body is independent of this route of administration, *i.e.*, it is not absorption rate limited.

The mean ±*SD* AUC, CL_o, and percent urinary recovery of intact bumetanide for the oral solution were 64 ± 27 ng·h/mL, 299 ± 100 mL/min, and 57 ± 12%, respectively (Table II). The corresponding values for the tablet were 66 ± 28 ng·h/mL, 310 ± 165 mL/min, and 58 ± 7%. Comparison of the respective areas and urinary excretion values between the two oral formulations showed a mean (tablet-solution) ratio of 1.0 (Table II), indicating that the extent of absorption of bumetanide from the tablet and solution oral dosage forms are equivalent, and that galenic availability was not a problem for the oral tablet. The similar apparent oral clearance values for both oral formulations further supports the assumption of complete absorption of bumetanide from the tablet. However, urinary excretion of intact bumetanide indicated absolute bioavailabilities of 0.84 and 0.86 for the oral solution and tablet, respectively, when compared with the intravenous dose. The respective bioavailabilities calculated from the AUCTr values were 0.74 and 0.78. Since the fraction of the dose excreted in the urine as intact bumetanide is large and the elimination of the drug from the plasma is fast, the urinary excretion method of estimating bioavailability is probably a more reliable method for bumetanide. A recent study (12), however, showed that there was no difference in the bioavailability values calculated by either the AUC or the urinary excretion method (0.90 and 0.89, respectively).

The diminished availability of intact drug to the systemic circulation following oral admninistration tends to suggest that bumetanide undergoes first-pass clearance. In support of this observation, the first-pass metabolism

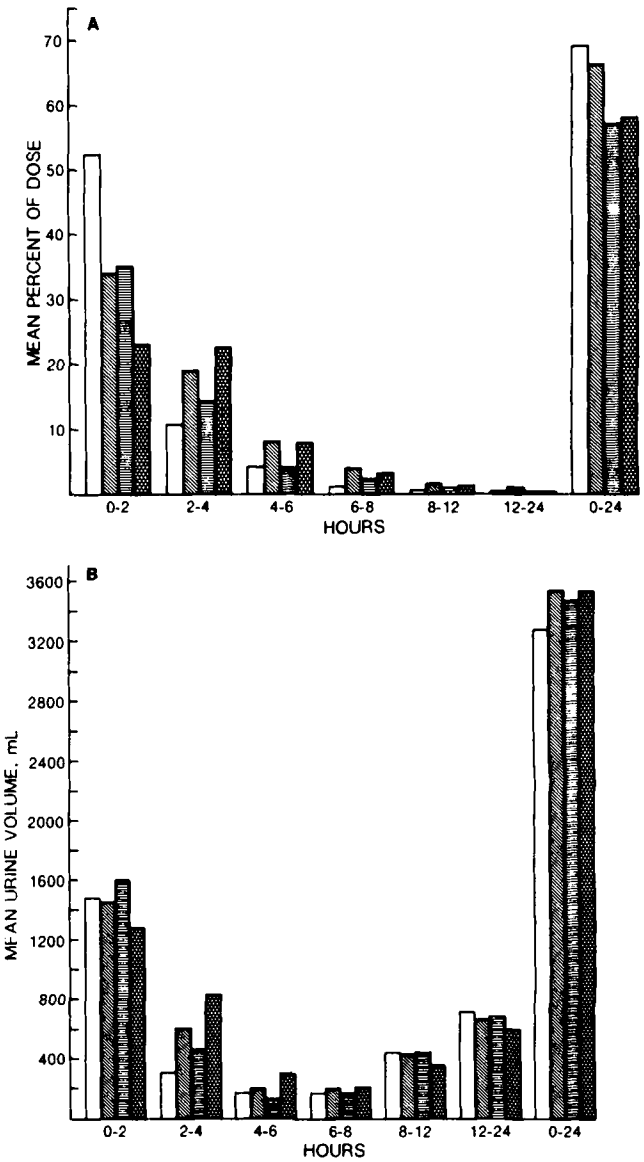

Figure 3—*Mean urinary excretion of intact bumetanide (A) and mean urine volume (B) following administrations of 1 mg of bumetanide to 12 normal subjects. Key: (□) intravenous; (▨) intramuscular; (▤) oral solution; (■) oral tablet.*

predicted for an oral dose, assuming linear kinetics, would be 10–20% (20). First-pass metabolism would also account for the higher mean oral clearance observed for both solution and tablet relative to the mean plasma clearance after intravenous and intramuscular administrations. Since ~70% of a parenteral dose and 60% of an oral dose are excreted as intact drug in the 0–24-h urine samples, the 10% difference as a function of the oral route of administration is consistent with the predicted limited extent of first-pass metabolism of the oral dose. These findings are consistent with the results of previous studies (10, 12), which have reported urinary recoveries for intact bumetanide of 60 ± 3% (10) and 66 ± 3% (12) from the intravenous dose, but only 49 ± 5% (10) and 59 ± 3% (12) from the oral dose.

The mean urinary excretion of intact bumetanide and the corresponding mean urinary volume following the four treatments are shown in Fig. 3. The diuretic effect of bumetanide following 1-mg iv, im, oral solution, and oral tablet doses was clearly evident in the first 2-h period, with mean urine volumes ranging from 1270 mL (tablet) to 1600 mL (oral solution). The diuretic activity lasted for ~4–8 h for all treatments, and the mean cumulative diuresis after 24 h was the same for the four treatments. The diuresis profile of bumetanide parallels the urinary excretion profile of intact bumetanide in that the major portion of intact bumetanide was excreted during the first 2 h following drug administration (Fig. 3). This confirms previous observations (9, 21) that the diuretic activity of bumetanide is closely associated with the concentration of intact drug in the kidney. The fact that the oral formulations are as effective diuretics as the parenteral preparations presumably reflects the maintenance of a minimum effective plasma or renal tubule concentration of bumetanide (22).

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Determination of Total Captopril in Human Plasma by Gas Chromatography–Mass Spectrometry with Selected-Ion Monitoring After Reduction of Disulfides

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Abstract □ Captopril is liberated from covalently protein-bound disulfides and other disulfide metabolites in human plasma by reduction with tri-*n*-butyl-phosphine. The captopril is then treated with *N*-ethylmaleimide, purified on XAD-2 resin, eluted with ethyl acetate, and methylated prior to its determination by gas chromatography–mass spectrometry with selected-ion monitoring. The limit of detection is 20 ng/mL of plasma.

Keyphrases □ Captopril—reduction of disulfides, GC–MS, selected-ion monitoring □ GC MS—selected-ion monitoring, total captopril in human plasma, reduction of disulfides

Captopril (I), an orally active angiotensin I-converting enzyme inhibitor (1, 2), is currently marketed for the treatment of hypertension (3, 4). Because of the extreme reactivity of thiols in biological systems, captopril is quantitatively converted to a derivative, such as the *N*-ethylsuccimide derivative (II), prior to analysis (5). The reaction product was determined by a gas chromatographic–mass spectrometric (GC–MS) method (6, 7) with selected-ion monitoring for captopril in whole blood and by a radiometric-thin-layer chromatographic (RTLC) procedure for establishing the biological disposition of captopril (8, 9). The latter method has been used in various metabolism and pharmacokinetic studies (10–12). Other reported methods for the determination of captopril employ GC (13), HPLC (14–17), and most recently GC–MS with selected-ion monitoring (18).

Metabolites of captopril identified so far include captopril disulfide (III), the mixed cysteine and glutathione disulfides of I (IV and V, respectively), and the *S*-methyl (VI) and *S*-methyl sulfoxide (VII) metabolites (9, 10, 12, 19). A substantial proportion of the captopril radioactivity in human blood after oral administration of radioactively labeled I was associated with the plasma fraction (8), which was covalently bound as plasma protein disulfides. It is believed that the plasma proteins and mixed disulfides with endogenous thiol-

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