Determination of Captopril in Human Blood and Urine by GLC–Selected Ion Monitoring Mass Spectrometry after Oral Coadministration with Its Isotopomer

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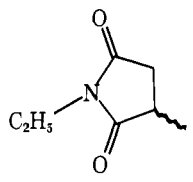
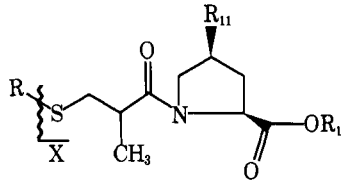
Abstract □ A modified electron-impact GLC-selected ion monitoring mass spectrometric method for captopril is described. Positive chemical ionization GLC-selected ion monitoring and direct chemical ionization confirms the specificity of this procedure for captopril and establishes the chemical ionization techniques as potential analytical methods. This procedure has been adapted to the simultaneous measurement of captopril and its isotopomer. The results of a pilot oral bioavailability study of four subjects receiving either 100 mg of captopril as a direct compression tablet or a solution concomitantly with a 100-mg solution of isotopomer is discussed.

Keyphrases □ Captopril—determination in human blood and urine, GLC-selected ion monitoring mass spectrometry, oral coadministration with isotopomer □ GLC-selected ion monitoring mass spectrometry—determination of captopril in human blood and urine, oral coadministration with isotopomer □ Urine, human—determination of captopril in urine blood, GLC-selected ion monitoring mass spectrometry, oral coadministration with isotopomer

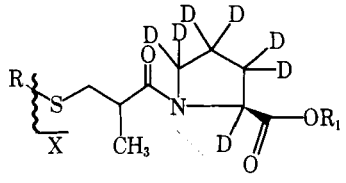
Captopril (I) is an orally active inhibitor of the angiotensin-converting enzyme (1, 2). A specific determination of I was performed by GLC–selected ion monitoring mass spectrometry (3). The succinimide (I*a*) was formed in whole blood by the addition of *N*-ethylmaleimide (IV), which was then adsorbed onto XAD-2 resin, eluted, methylated, and measured as the ester (I*b*). The collaborative studies obtained by the GLC selected ion monitoring and radiometric-TLC methods (4) established a high degree of specificity for the determination of the administered compound, in the presence of other captopril biotransformation products (5). Other recent assays for captopril in blood and urine employ GC (6) and high-performance liquid chromatography (HPLC) (7, 8).



$C_6H_8NO_2$



I, I*a*, I*b*, III, III*a*, III*b*



II, II*a*, II*b*

		R	R_I	R_{II}	MW	X EI	MH^+ PCI
Captopril	I	H	H	H	217		
	I *a*	$C_6H_8NO_2$	H	H	342		
	I *b*	$C_6H_8NO_2$	CH_3	H	356	230	357
Isotopomer	II	H	H	—	224		
	II *a*	$C_6H_8NO_2$	H	—	349		
	II *b*	$C_6H_8NO_2$	CH_3	—	363	237	364
Fluoroanalog	III	H	H	F	235		
	III *a*	$C_6H_8NO_2$	H	F	360		
	III *b*	$C_6H_8NO_2$	CH_3	F	374	248	

BACKGROUND

Based on the experience gained from the analysis of blood samples from more than 200 normotensive subjects, a number of modifications of the method (3) evolved to achieve more ease and reliability in processing samples as well as increased precision in making measurements. Currently, 1000 samples, typically collected in one multiple crossover bioavailability study, are processed monthly.

This highly specific and precise analytical method has shown the variability that exists in the disposition of the drug by individual subjects. Using the statistical criteria, a significant subject population is required to demonstrate bioequivalence of formulations. A number of investigators have successfully employed the coadministration of a drug with an isotopomeric formulation in absolute (9) and relative bioavailability (10–15) studies with fewer subjects and at a substantial savings in expense and time. Use of coadministered formulations places more emphasis on a comparison of intra- and intersubject variabilities and at the same time provides data better suited to the evaluation of pharmacokinetic parameters (16).

EXPERIMENTAL

Reagents—Captopril (I, 1-[*S*(2)-mercapto-2-methylpropionyl]-L-proline, CAS registry 62571-86-2), its heptadeutero-isotopomer(II) and 1-[(*S*,4*S*)-4-fluoro-1-(D-3-mercapto-2-methyl -1- oxopropyl)]-L-proline (III) were pharmaceutical grade materials[1] used with additional purification. Methanol, *N*-ethylmaleimide (IV), hydrochloric acid, sodium bicarbonate, diethylenetriaminetetraacetic acid[2] (V), citric acid, oxalic acid dihydrate, sodium phosphate dibasic ($Na_2HPO_4 \cdot 7H_2O$), ammonium phosphate dibasic, instant methanolic hydrochloric acid kit[3], 80–200 mesh Brockman Activity 1 neutral alumina, and sodium chloride were used without purification. Purified XAD-2 resin[3] was conditioned as previously described (3). Ethyl acetate was purified immediately before use by passing 600 ml through a neutral alumina 2.5 × 40-cm column to remove oxidizing impurities (3). Preparation of the phosphate buffer (pH 7.0) and methanolic hydrochloric acid have been previously described (3).

The solutions used for processing urine samples were prepared by a previous method (17). The 0.8% V solution was prepared by dissolving 4 g of V in 500 ml of 0.08 *M* NaOH, sonicated for ~30 min in an ultrasonic bath. The 0.08 *M* NaOH solution was prepared by dissolving 3.2 g in 1 liter of distilled water. The citric acid–oxalic acid solution was prepared by dissolving 105 g of citric acid monohydrate and 63 g of oxalic acid in 1 liter of distilled water.

Blood and Urine Collection—From 12 to 15 ml of blood was drawn[4] and processed as previously described (3) by the addition of 75 mg of IV to each sample. Frozen citrated whole blood was used for calibration and control samples. Urine samples were collected according to a previous

[1] E. R. Squibb & Sons, Princeton, N.J.
[2] G. F. Smith Chemical Co., Columbus, Oh.
[3] Applied Science Lab. Inc., State College, Pa.
[4] Becton-Dickinson, Vacutainer.

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Table I—Summary of Calibration Data GLC–Selected Ion Monitoring Mass Spectrometry

Sample Type	Analyte		Ion m/z	Internal Reference Standard		Ion m/z	n	Intercept I_n	Slope k_n
Blood	Captopril	I	230.1	Isotopomer	II	237.1	1	0.0192	1.2991
	Captopril	I	230.1	Fluoroanalog	III	248.1	2	0.0275	2.0463
	Isotopomer	II	237.1	Fluoroanalog	III	248.1	3	0.0047	1.7396
Urine									
High	Captopril	I	230.1	Fluoroanalog	III	248.1	2	0.0911	1.9316
	Isotopomer	II	237.1	Fluoroanalog	III	248.1	3	0.0595	1.7111
Low	Captopril	I	230.1	Fluoroanalog	III	248.1	2	0.0157	2.3417
	Isotopomer	II	237.1	Fluoroanalog	III	248.1	3	0.009	2.0428

Table II—Comparison of Captopril Blood Levels Determined by Electron-Impact and Chemical Ionization GLC–MS

Time, hr	Captopril Blood Levels, ng/ml Subject A EI	CI	Diff, Δ EI–CI	Captopril Blood Levels, ng/ml Subject B EI	CI	Diff, Δ EI–CI
0.0	2.3	7.4	−5.1	4.1	1.7	2.4
0.25	21.1	26.1	−5.0	59.8	61.5	−1.7
0.50	272.0	265.0	7.0	318.0	317.0	1.0
0.75	432.0	437.0	−5.0	450.0	433.0	17.0
1.0	375.0	378.0	−3.0	337.0	344.0	−7.0
1.5	186.0	176.0	10.0	165.0	148.0	17.0
2.0	104.0	98.3	5.7	69.9	81.7	−11.8
3.0	31.4	28.4	3.0	20.4	20.9	−0.5
4.0	21.1	14.8	6.3	10.3	10.3	0.0
6.0	7.8	16.6	−8.8	6.4	5.6	0.8
8.0	1.8	1.6	0.2	2.2	2.9	−0.7
12.0	0.8	2.2	−1.4	2.0	1.8	0.2

procedure (17) for the colorimetric determination of captopril. The urine for each subject was collected over the prescribed time period, and 5 ml of refrigerated solution was added to the temporary container. Each subject's sample was swirled and added to the permanent container which contained 100 ml of citric acid–oxalic acid solution. The permanent container was returned to a 5° refrigerator, and at the end of the collection period the pH was tested with pH 2–4 paper. An additional 100 ml of citric acid–oxalic acid solution was added if the pH exceeded 2. Each volume of urine and citric acid–oxalic acid solution added to the container was measured and recorded, and 50–100 ml of this solution was transferred to a smaller container for storage in a refrigerator.

Before an elapsed time of 48 hr, 0.2 ml of a solution of IV (5 g/ml in acetone) was added to 5 ml of refrigerated urine sample, followed by 5 ml of ammonium phosphate dibasic solution (250 g/liter in water). After standing for 15 min, the solution was sampled for analysis or frozen if the analysis was to be performed at a later date.

Extraction of Blood Samples—The extraction procedure remained essentially the same (3), except that the samples were processed in batches of 11 plus one control. For samples containing the isotopomer, 2.5 μg of III was added as an internal reference to each 5-ml aliquot of blood sample, while 2.5 μg of II was added as the internal reference for the measurement if only captopril was administered. For coadministration studies, the control was composed of 5 μg each of I and II and 2.5 μg of the internal reference III. For the single administration samples, the control sample was 5 μg of I and 2.5 μg of the internal reference II. The samples were methylated after extraction, and just prior to measurement they were reconstituted with 20 μl of acetone.

Table III—Comparison of Captopril Blood Levels Determined by Electron-Impact GLC–MS and Direct Chemical Ionization

Electron Impact GLC–MS, ng/ml	Direct Chemical Ionization, ng/ml	Diff, GLC–MS–Direct Chemical Ionization
0.0	5.8	−5.8
2.3	2.5	−0.2
5.4	5.8	−0.4
9.6	1.3	8.3
13.3	13.8	−0.5
15.0	0.6	14.4
30.5	26.7	3.8
84.6	81.9	2.7
77.1	78.1	−1.0
196.0	180.0	16.0
213.0	204.0	9.0
312.0	303.0	9.0
408.0	417.0	−9.0
430.0	430.0	0.0
447.0	443.0	4.0
500.0	479.0	21.0
642.0	621.0	21.0

Extraction of Urine Samples—Urine samples collected just prior to and 4 hr after administration were processed in the same manner as the blood samples (3). To each 3-ml aliquot, 0.1 ml of the fluoro internal reference III containing 2.5 μg was added.

Samples collected during the first 4 hr after administration, were processed by adding 0.1 ml of internal reference containing 25 μg of internal reference III to a 2-ml aliquot of urine sample. The control urine sample contained 50 μg each of I and II and 25 μg of III. To each 150-mm screw-capped culture containing the urine sample, 2 g of sodium chloride and 2 ml of 6 N H_3PO_4 were added, the tubes covered with caps, and agitated on a shaker for 5 min. Five milliliters of purified ethyl acetate was added, the tubes were shaken for 5 min, and then centrifuged at 2000 rpm for 5 min. Five-tenths milliliter of the ethyl acetate layer (top) was then transferred to a 1-ml reaction vial[5] and the solvent was evaporated under nitrogen[6]. The contents of the reaction vials were then further dried under vacuum at room temperature for 15 min in an oven or dessicator. The samples were then methylated as described (3) and stored in a freezer. Just prior to measurement, the samples were dissolved in 20 μl of acetone.

Calibration and Preparation of Control Blood and Urine Samples—Varying amounts of I (and an equal amount of II for simultaneous measurements) were added to 5 ml of citrated blood (containing 25 mg of IV, in a 150-mm screw-capped culture tube), to yield blood concentrations of 0–1500 ng/ml. For the simultaneous measurements of captopril and its isotopomer, 0.1 ml of solution containing 2.5 μg of internal reference III was added, while an equivalent amount of internal reference II was added if only I was to be measured. The control sample represents the calibration point of 1000 ng of analyte(s) and 500 ng of internal reference per milliliter of blood.

The standard curve for the low-concentration urine samples was prepared from 3-ml urine samples containing 0–7.5 μg of I and II and 2.5 μg of internal reference III, which were processed as described for urine samples collected during the first 4 hr after administration. Control urines, containing either 5 or 50 μg of analytes I and II and 50% of internal reference III were processed for either low or high concentrations of captopril.

[5] Hewlett-Packard, Palo Alto, Calif.
[6] Mini-Vap.

Table IV—Statistical Evaluation of Captopril Blood Calibration Data

Ratio ng Captopril I / ng Isotopomer II[a]	$Int_{m/z\ 230}/Int_{m/z\ 237}$ Mean[b]	Standard Deviation (s)	s/Mean
0.0	0.01915	0.002783	0.145
0.0625	0.09775	0.01099	0.112
0.125	0.19146	0.02515	0.131
0.25	0.34287	0.01893	0.055
0.50	0.68678	0.02410	0.035
1.0	1.3050	0.04248	0.033
2.0	2.5694	0.1344	0.052
3.0	3.8928	0.1659	0.043

[a] 2500 ng internal reference standard added. [b] Based on nine measurements.

The ratio of the maximum peaks heights, R_n, was plotted *versus* the respective nanogram ratio R'_n:

n	R_n	R'_n
1	$I_{m/z\ 230}/I_{m/z\ 237}$	1/2
2	$I_{m/z\ 230}/I_{m/z\ 248}$	1/3
3	$I_{m/z\ 237}/I_{m/z\ 248}$	2/3

The intercept, I_n, is the ratio R_n for the blank extract ($R'_n = 0$). Linear regression:

$$R_n = I_n + k_n R'_n \qquad \text{(Eq. 1)}$$

establishes the slopes, k_n, and the intercepts I_n for Eq. 1 (Table I).

Selected Ion Monitoring—From 1 to 2 μl of solution, added to the tip of a GLC solids injector[7], was allowed to evaporate. The appropriate ion profiles (m/z 230.1, m/z 237.1, m/z 248.1) were obtained by selected ion monitoring. The maximum peak heights were measured using a program written especially for the batch processing of the data[8]. The program selects the baseline-corrected maximum peak heights of the requested ions. The mean ratio, R''_n, and standard deviation for three to five injections of the control sample bracketing the samples were determined for subsequent use to correct the data for differences in response from the calibration slope, k_n. The adjustment factor, A, is related to the calibration slope and the measured ratio of the control, R''_n, by Eq. 2:

$$A = \frac{R''_n}{I_n + 2k_n} \qquad \text{(Eq. 2)}$$

The measured ratio of the sample, R^*_n, is related to R_n by Eq. 3:

$$R_n = R^*_n/A \qquad \text{(Eq. 3)}$$

which is then used to calculate the ng (or μg) ratio of analyte to internal reference, R'_n:

$$R_n = R^*_n/A = I_n + k_n R'_n \qquad \text{(Eq. 4)}$$

The concentration of analyte, C_x, is calculated from Eq. 5:

$$C_x = R'_n(F/M) \qquad \text{(Eq. 5)}$$

where C_x is the concentration of analyte in nanograms per milliliter (blood) or micrograms per milliliter (urine), F is the quantity of internal reference (ng for blood; μg for urine) added to M milliliters of sample, and R'_n is the ratio of analyte to internal reference calculated from Eq. 1.

Instrumental Procedure—The combined GLC–MS with data system[9] was operated under selected ion monitoring mode. The instrument was tuned[10] for electron-impact (EI) MS from 69 to 502 Daltons using perfluorotributylamine at an ionization voltage of 70 eV and at an emission of 300 μA. The ion source was controlled at a constant temperature of 200°.

A septum guide[11] was attached to the GLC injector. The analyses were carried out on an 80- cm × 2-mm i.d. silanized glass column packed with 3% OV-101[12] which was connected through a single-stage jet separator.

[7] Scientific Glass Equipment Co., SI-1RDS, Penzias Assoc., Roslyn Heights, N.Y.
[8] The BASIC program for the HP 5985B GCL–MS is available upon request.
[9] Hewlett-Packard, Model 5985B, Palo Alto, Calif.
[10] AUTOTUNE.
[11] Supelco, Cat. No. 2-0839, Bellefonte, Pa.
[12] On 80/100 Supelcoport, Supelco, Bellefonte, Pa.

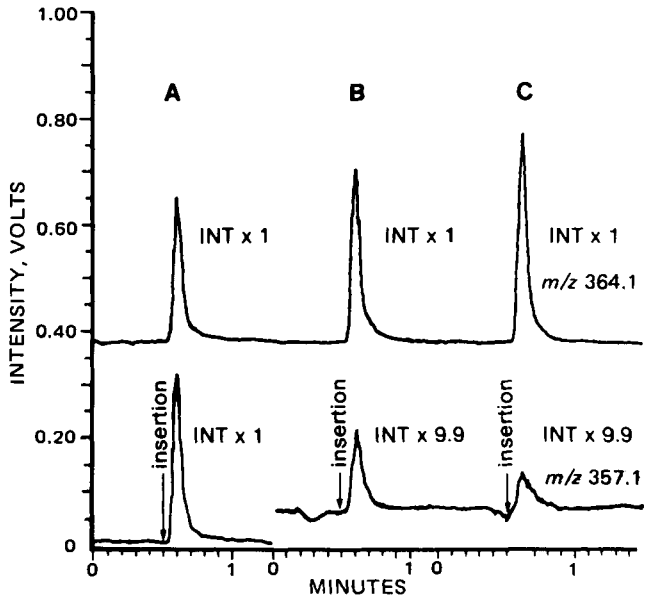

Figure 1—*Direct chemical ionization of captopril blood samples containing 500 ng/ml of internal reference as IIb and (A) 442, (B) 13.8, and (C) 2.5 ng/ml of captopril as Ib*

The separator and transfer line were maintained at 275° while the column was kept at an isothermal temperature of 260° and the injector was heated to 290°. A typical retention time of 0.6 min was obtained at a helium flow rate of 30 cm³/min. Slight adjustments in the column temperature were made to keep the retention time within the desired time window, when necessary. Helium, otherwise diverted, was allowed to flow into the ion source from 0.1 to 1.3 min after sample injection. The multiplier and electron voltages were only applied during the data collection period from 0.3 min until the termination of the GLC run. When required, portions of the column packing or the entire column was replaced to maintain optimum chromatographic conditions. The septum was replaced each day.

Positive chemical ionization GLC–MS was obtained with methane as the GLC carrier gas, at a flow rate of 20 cm³/min, directly into the source to create a 1-torr source pressure. Otherwise, the chromatographic conditions were identical to that of the electron impact-GLC–MS.

Direct chemical ionization mass spectra and measurements were taken on a modified quadrupole mass spectrometer[13] equipped with a data acquisition system[14]. The respective emission and electron energy were 1000 μA and 300 V. Resolution, sensitivity, and mass calibration were made according to the recommended procedures. Water was employed as the reagent gas, admitted from behind the sample and from a separate line into the chemical ionization source. The selected ion monitoring data were recorded on the MH^+ at a nominal source pressure of 1 torr and a source temperature of 250°. For the direct chemical ionization experiments ~1 μl of solution was deposited on a quartz probe.

RESULTS AND DISCUSSION

While the extraction procedure remains essentially unchanged (3), organizational efficiencies permitted each of four technicians to extract daily 11 samples plus the control. The sample data collection procedure was modified to increase the daily capacity to approximately twice the number of samples extracted. An isothermal column, a solids sample injector, and a retention time of the analyte of 0.6 min allowed sample injections at the rate of 12–15/hr. There was also a noticeable decrease in the background from the elimination of the solvent. Otherwise diverted by a dump valve, the helium carrier gas flowed into the source only during data acquisition to reduce column bleed. Instrument maintenance was also reduced by operating the ion source and multiplier only while collecting data.

Because the manual peak height measurement routine provided with the instrument was time consuming, a program was written to scan each data file, determine the baselines, the net peak heights for each acquired ion, and calculate the peak height ratios and concentrations. As elabo-

[13] SIMULSCAN, Extranuclear Corp., Pittsburgh, Pa.
[14] Model 69KD, Technivent Corp., St. Louis, Mo.

Table V—Comparative and Relative Bioavailability Parameters of 100-mg Direct Compression Tablet and Solution Formulations of Captopril Coadministered with Isotopomer Solution

Parameter	Mean Bioavailability Parameter: Direct Compression Tablet[a] Captopril	Solution[b] Isotopomer		Relative[c] Parameter
C_{max}, μg/ml, Mean[e]	0.931	0.678	C_{m1}	1.59
⟶ (± *SEM*)	(0.156)	(0.180)	C_{m2}	(0.38)
T_{max}, hr Mean	0.63	0.63		1.0
(±*SEM*)	(0.07)	(0.07)		(0)
$AUC_{0\rightarrow12}$ hr Mean	1.209	0.903	AUC_1	1.59
(± *SEM*)	(0.099)	(0.187)	AUC_2	(0.44)
	Solution[d] Captopril	Solution[b] Isotopomer		
C_{max}, μg/ml Mean	0.790	0.753	C_{m1}	1.06
(± *SEM*)	(0.054)	(0.052)	C_{m2}	(0.07)
T_{max}, hr Mean	0.75	0.81		0.94
(± *SEM*)	(0)	(0)		(0.06)
$AUC_{0\rightarrow12}$, hr Mean	1.065	1.061	AUC_1	1.01
(± *SEM*)	(0.065)	(0.039)	AUC_2	(0.07)

[a] Direct compression tablet (100 mg) coadministered. [b] Solution of isotopomer (100 mg) coadministered. [c] Parameter direct compression tablet (or solution)/parameter isotopomer. [d] Solution (100 mg) coadministered. [e] For four subjects.

rated in the experimental section, multiple measurements of the control sample allows for the single-point calibration to increase the accuracy of the measurements. It is also essential to keep the ion source at a controlled temperature to achieve the most precise measurements. Changes in the control ratio value may result from contaminant deposits on the source and quadrupole rods. In the case of the low-concentration urine extracts, the repeller was frequently shorted out because of the extraneous materials present in the extract. When this occurred, a sudden loss in sensitivity and a drastic change in the value of the control ratio was noted. While not capable of the same limit of detection, the extraction procedure for the higher concentration urine is preferred.

The specificity of the measurements of captopril was independently validated using positive chemical ionization GLC–selected ion monitoring by measuring the MH^+ of the reference (II*b*) and analyte (I*b*) derivatives. A comparison with the electron-impact data of two subjects demonstrates good correlation between the two data sets (Table II) reconfirming the specificity of the electron-impact method and also qualifying the positive chemical ionization GLC–selected ion monitoring procedure as an alternate method.

A limited set of data compares the electron-impact GLC–selected ion monitoring data with results obtained by direct chemical ionization. Nanogram quantities of a sample deposited on the direct insertion probe produce readily measureable intensities (Fig. 1) that can be used in the quantitative measurement of captopril related compounds (Table III).

The limit of detection and sensitivity of the method using the isotopomer as the internal reference was established (18) (Table IV). Three sets of calibration extracts were each measured three times to provide a total of nine measurements per calibration point. The weighted regression was chosen because the ratio of the standard deviations (s) to respective mean varies over the concentration range. From 0 to 60 ng/ml, the coefficient of variation (CV) is 13%. The 3s value of the lowest calibration point puts the limit of detection at 13 ng/ml (99% confidence limit). The actual distribution of 108 zero-hour samples has two-thirds of the values at ≤6 ng/ml and 95% of the values at ≤14.5 ng/ml (Fig. 2), in good agreement with the value derived from the detection limit of the lowest nonzero calibration 3s value. The limit of detection and the sensitivity could have been enhanced if a low level calibration curve were employed using one-tenth the amount of internal reference. No significant benefit would have been derived for these bioavailability studies, however.

Table VI—Comparative Bioavailability Parameters of 100-mg Direct Compression Tablets and Solution Formulations of Captopril

Parameter	Direct Compression Tablet[a] Captopril	Solution[b] Captopril
C_{max}, μg/ml Mean[c]	0.90	0.82
(SEM)	(0.05)	(0.05)
T_{max}, hr Mean	0.74	0.84
(SEM)	(0.04)	(0.07)
$AUC_{0\rightarrow12}$, hr Mean	1.23	1.16
μg × hr/ml (*SEM*)	(0.05)	(0.04)

[a] Captopril (100 mg) as a direct compression tablet. [b] Captopril (100 mg) as a solution. [c] For 18 subjects.

The relative standard deviation (RSD)[15] of 53 pairs of duplicate extracts of 0.75-hr blood samples is 3.9%, which is in good agreement with the CV ≤5.5% calculated from the 125 to 1500 ng/ml calibration data.

Because of intra- and intersubject variation in the disposition of drugs, a significant number of subjects, usually ≥16, are required to demonstrate bioequivalence in a multiple crossover study. Using simultaneous administration with isotopomeric formulations (9–15), superior bioavailability data may be obtained with fewer subjects. A pilot two-way crossover study was performed to compare the bioavailability of a 100-mg direct compression tablet with a 100-mg solution of I, given orally, along with a coadministered solution of 100 mg of the isotopomer, II.

The mean bioavailability parameters determined for the tablet and oral solution given to four subjects in this study compare favorably (Table V) with data obtained from 18 subjects administered in the usual way (Table VI). No significant difference was found for the mean bioavailability data derived from the coadministration of captopril and isotopomer as solutions (Tables V and VII). Consequently, the relative bioavailability parameters, C_{m1}/C_{m2} and AUC_1/AUC_2[16] are approximately in unity. However, the relative bioavailability parameters, C_{m1}/C_{m2} and AUC_1/AUC_2, for the direct compression tablet and isotopomer solution are significantly different. Examination of the data (Table VIII) reveals the captopril blood levels of subject 1 were approximately three times

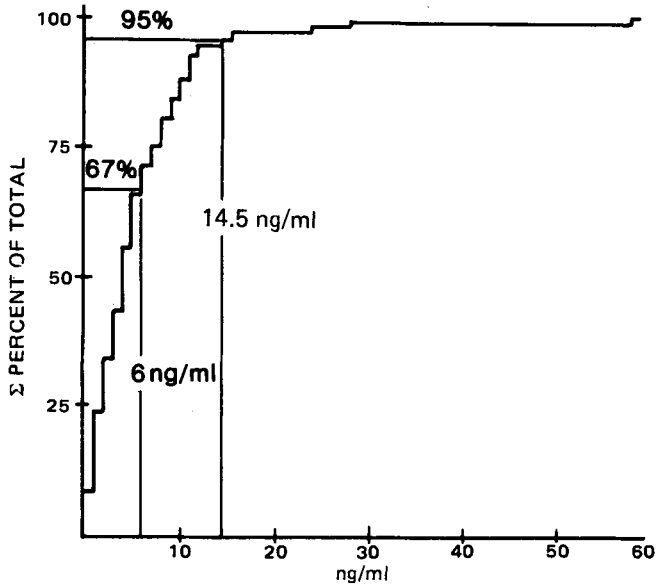

Figure 2—*Distribution of measured values of 108 zero-hour blood samples as a percent of total number of samples: maximum concentrations containing 67 and 95% of values are shown.*

[15] $RSD = (\Delta/M) \times 100$; $\Delta = (Ext_1 - Ext_2)$, $M = (Ext_1 + Ext_2)/2$.

[16] C_{m1}/C_{m2}—relative maximum concentrations of captopril (I) and isotopomer (II) AUC_1/AUC_2–relative area under the curve of I and II.

Table VII—Concentrations of Captopril and Isotopomer Each Sampling Time—Solution *versus* Solution

Time, hr	Captopril Solution[a], ng/ml Subject No. 1	2	3	4	Mean	Isotopomer Solution[b], ng/ml Subject No. 1	2	3	4	Mean
0.25	50	175	239	197	165	50	121	208	125	126
0.5	281	637	761	550	557	311	573	674	466	506
0.75	714	788	944	716	790	834	727	828	612	750
1.0	554	755	698	648	664	708	738	630	591	667
1.5	314	327	377	336	339	408	324	346	322	350
2.0	169	239	159	176	186	214	240	146	165	191
3.0	48	58	53	69	57	66	61	48	67	61
4.0	24	27	18	29	25	32	27	21	27	27
6.0	10	11	6	11	10	14	12	8	13	12
8.0	9	5	0	4	5	10	7	2	6	6
12.0	0	5	0	1	2	4	5	0	2	3

[a] Captopril (100 mg) coadministered as a solution. [b] Isotopomer (100 mg) coadministered as a solution.

Table VIII—Blood Concentrations of Captopril and Isotopomer at Each Sampling Time—Tablet *versus* Solution

Time, hr	Direct Compression Tablet[a], ng/ml Subject No. 1	2	3	4	Mean	Isotopomer Solution[b], ng/ml Subject No. 1	2	3	4	Mean
0.25	83	157	361	299	225	43	131	321	230	181
0.5	424	455	991	1322	806	199	360	892	1068	630
0.75	840	572	969	962	836	310	440	856	824	608
1.0	827	497	604	785	678	277	415	541	656	472
1.5	411	308	291	395	351	126	260	270	327	246
2.0	139	278	133	183	183	59	242	126	175	151
3.0	67	81	76	65	72	23	76	71	61	58
4.0	31	29	38	33	33	9	28	42	33	28
6.0	13	8	11	11	11	4	9	12	10	9
8.0	6	1	6	18	8	1	6	8	6	5
12.0	1	0	0	1	1	0	0	3	3	2

[a] Captopril (100 mg) coadministered as a direct compression tablet. [b] Isotopomer (100 mg) coadministered as a solution.

Table IX—Urinary Excretion of Captopril and Isotopomer as a Percent of Dose

Time, hr	Captopril Direct Compression Tablet[a], % of Dose Subject No. 1	2	3	4	Isotopomer Solution[b], % of Dose Subject No. 1	2	3	4
0–4	22.0	18.3	78.4[c]	22.8	8.0	14.6	72.4[c]	21.7
4–8	2.4	3.0	1.7	4.2	0.87	2.6	1.5	3.7
8–12	0.67	0.63	0.31	0.28	0.22	0.51	0.30	0.28
Total 0–12	25.1	20.3	—	27.3	9.1[d]	17.7	—	25.7
	Captopril Solution[e], % of Dose Subject No. 1	2	3	4	Isotopomer Solution[b], % of Dose Subject No. 1	2	3	4
0–4	19.2	24.3	8.2	8.2	22.9	23.1	7.2	7.4
4–8	3.3	1.5	1.7	2.7	4.3	1.5	1.5	2.4
8–12	0.38	0.45	0.29	0.48	0.58	0.51	0.28	0.54
Total 0–12	22.9	26.3	10.2	11.4	27.8	25.1	8.7	10.3

[a] Captopril (100 mg) coadministered as a direct compression tablet. [b] Isotopomer (100 mg) coadministered as a solution. [c] Urine appears to be in error. [d] Total isotopomer excreted from 0–12 hr is ~ one-third of excreted captopril for this subject. [e] Captopril (100 mg) coadministered as a solution.

greater than the isotopomer levels, as were the levels of compounds excreted in urine (Table IX). Significantly, this difference would have gone undetected in a conventional study. If the data for this subject are eliminated, the relative bioavailability parameters, while not in unity, are significantly lower. Previous bioavailability studies have also shown that a small difference in bioavailability parameters exists between direct compression tablet and solution formulations (Table VI).

The statistical evaluation of the coadministration bioavailability data was made to determine the power of the method to meet criteria for establishing differences in bioavailability. Excluding the suspect data of subject 1, data from only two of the three remaining subjects would be sufficient to establish differences in bioavailability that might require 14–40 subjects in a conventional study. However, from a practical point of view, it would seem more appropriate to expand such a study as this to ~6 subjects to provide a wider range of disposition, which would still result in a substantial savings in clinical and laboratory expense.

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Pharmacokinetics of Procainamide in Rats with Extrahepatic Biliary Obstruction

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Abstract □ The pharmacokinetics of the widely used antiarrhythmic agent, procainamide, was studied in rats with extrahepatic biliary obstruction produced by ligation of the common bile duct. Various biological fluids, including plasma, saliva, and urine, were analyzed for procainamide and/or its major metabolite, *N*-acetylprocainamide. Ligation of the common bile duct immediately prior to intravenous administration of 50 mg/kg procainamide did not alter plasma, saliva, or urine concentrations of procainamide, indicating that biliary excretion was of minor importance in the elimination of procainamide. However, bile duct ligation allowed to persist for 4 days significantly elevated plasma, saliva, and urine levels of procainamide. While the increase in urinary procainamide paralleled the increase observed in plasma, salivary concentrations did not. Bile duct ligation did not appear to impair nonmicrosomal acetylation of procainamide, although a significantly greater amount of unchanged drug was found in the urine after 24 hr. Pharmacokinetic analysis *via* the two-compartment open model showed that bile duct ligation caused a decrease in overall clearance from ~61.94 to 28.71 ml/kg/min. This reduction probably resulted from the decreased microsomal metabolism of procainamide. The significant reduction in the apparent volume of distribution from 3.76 to 2.72 liter/kg could be the result of reduced binding sites. There was also a significant increase in the elimination half-life of procainamide from 47.39 to 78.64 min in bile duct ligated rats.

Keyphrases □ Pharmacokinetics—procainamide, rats with extrahepatic biliary obstruction □ Procainamide—pharmacokinetics in rats with extrahepatic biliary obstruction □ Biliary obstruction—extrahepatic, pharmacokinetics of procainamide in rats

Biliary stasis in humans can be caused by tumors, drugs, or various pathophysiological alterations. Many patients with biliary stasis are also critically ill with other serious conditions and therefore are under treatment with a number of pharmacological agents. Inhibition of bile flow and/or biliary excretion may have profound consequences on the pharmacokinetics of drugs, not only because bile can be a route of irreversible elimination, but also because cholestasis may affect microsomal drug metabolism. Tavolini and Guarino (1) demonstrated altered disposition of doxorubicin[1], an antineoplastic agent eliminated extensively in the bile. Other investigators (2–5) demonstrated that microsomal metabolism of a number of drugs that are not extensively excreted in bile is significantly decreased, thus prolonging the elimination half-life ($t_{1/2\beta}$). However, the effects of biliary stasis on the pharmacokinetics of drugs with minimal biliary excretion and minimal microsomal metabolism are unclear. The antiarrhythmic agent procainamide (I) is such a drug. It was the purpose of this investigation to study the effects of cholestasis, induced by mechanical obstruction of the common bile duct, on the pharmacokinetics of I. Salivary levels were measured as an index of drug distribution to the peripheral tissue compartment, and urine levels were assayed to obtain an estimate of the renal clearance of procainamide and the relative urinary metabolic profile.

[1] Adriamycin.

EXPERIMENTAL

Urine, saliva, and plasma were obtained from a group of normal Wistar rats (weight 190–260 g) for the quantitation of I. Rats were anesthetized with 50 mg/kg ip pentobarbital[2]. Initially, a tracheotomy was performed to facilitate breathing, and then the animals were prepared for collection of parotid saliva according to a previously described method (6). Stimulation of salivation was accomplished by infusing pilocarpine[3] (0.25 mg/ml) into the right brachial artery at a rate of 0.11 ml/min for 90 min. For the administration of I[4] (50 mg/kg), the femoral vein was cannulated. Urine was collected *via* cannulation of the ureters. Urine and saliva were collected over three 30-min collection periods, and 1.5 ml of whole blood was collected at 30, 60, and 90 min postinjection from a cannula placed in the femoral artery. From a separate group of normal rats, plasma was obtained at 30, 60, and 90 min following administration of 50 or 100 mg/kg of I for the quantitation of the acetylated metabolite, *N*-acetylprocainamide (II).

In anticipation of the fact that saliva was to be collected simultaneously with the other biological fluids, preliminary studies were performed to determine the effects of pilocarpine infusion of plasma procainamide concentration and urinary procainamide excretion. Ten control (no pilocarpine) and 10 test rats were used for plasma collection, and another group of five controls and five test animals were used for urine collection.

Extrahepatic biliary obstruction was produced in another group of

[2] Nembutal, Abbott Laboratories, North Chicago, Ill.
[3] Sigma Chemical Co., St. Louis, Mo.
[4] Squibb Institute for Medical Research, Princeton, N.J.

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