Journal of Pharmacokinetics and Biopharmaceutics, Vol. 9, No. 2, 1981

Evidence of Nonlinearity in Digoxin Pharmacokinetics

J. G. Wagner,[1,4] K. D. Popat,[2] S. K. Das,[2] E. Sakmar,[1] and H. Movahhed[3]

Received June 3, 1980—Final October 28, 1980

Six normal male volunteers received 0.5 mg label doses of digoxin as (a) a bolus intravenous injection over 2 min, (b) a constant rate intravenous infusion over 1 hr, (c) a constant rate intravenous infusion over 3 hr, and (d) a solution in 5% dextrose given orally. Plasma concentrations of digoxin were measured by radioimmunoassay for a 4 day period and urinary excretion for a 6 day period after the single doses. The mean (coefficient of variation) total areas under the plasma concentration-time curves per 0.5 mg of digoxin were (a) 35.55 (14.8%), (b) 30.20 (27.7%), (c) 25.80 (35.5%), and (d) 15.47 (49.9%); the means differed significantly ($0.01 > p > 0.005$). The mean (coefficient of variation) total amounts excreted in the urine as a fraction of the dose were (a) 0.689 (6.31%), (b) 0.517 (20.4%), (c) 0.588 (16.8%), and (d) 0.374 (23.4%); the means differed significantly ($p < 0.001$). Both the total clearance and the nonrenal clearance of digoxin differed significantly with the method of intravenous administration. The slower the rate of input of digoxin to the body, the greater were both the total clearance and the nonrenal clearance of the drug, which strongly suggests nonlinear pharmacokinetics.

KEY WORDS: digoxin bioavailability; digoxin radioimmunoassay; plasma concentrations and urinary excretion of digoxin; bolus injection and constant infusion.

This work was supported in part by National Institutes of Health Grant 1 R01 HL 23862-01 and in part by National Institutes of Health General Clinical Research Center Grant 5M01 RR421.

[1]College of Pharmacy and Upjohn Center for Clinical Pharmacology, The University of Michigan Medical Center, Ann Arbor, Michigan 48109.

[2]Department of Internal Medicine (Cardiology), The University of Michigan Medical Center, Ann Arbor, Michigan 48109.

[3]Department of Pharmacology and Upjohn Center for Clinical Pharmacology, The University of Michigan Medical Center, Ann Arbor, Michigan 48109.

[4]Address correspondence to Dr. John G. Wagner, Upjohn Center for Clinical Pharmacology, The University of Michigan Medical Center, Ann Arbor, Michigan 48109.

0090-466X/81/0400-0147$03.00/0

INTRODUCTION

The human study discussed in this article was designed to elucidate certain discrepancies in digoxin pharmacokinetics that were reported by other investigators. Greenblatt *et al.* (1) administered doses of 0.75 mg of digoxin by both rapid intravenous injection (over a 2 to 3 min period) and in 250 ml of 5% dextrose in water over a 1 hr period by means of a constant rate infusion pump to a panel of eight healthy males. They reported that (a) the mean 6 day cumulative urinary excretion of digoxin after rapid injection, 0.52 mg (69% of the dose), was significantly ($p < 0.001$) lower than that after 1 hr infusion, 0.57 (76%); (b) between subject variability in cumulative urinary excretion after rapid intravenous injection (coefficient of variation = 7.5% of the mean) was significantly ($p < 0.01$) greater than after 1 hr infusion (coefficient of variation = 2.2% of the mean); and (c) mean areas 0–8 hr under the serum concentration-time curves were not significantly different for the rapid injection and 1 hr infusion.

Marcus *et al.* (2) administered 0.4 mg doses of digoxin to five normal volunteers both as a constant rate infusion over 1 hr and as an infusion over 3 hr with 0.1 mg of the digoxin being infused during the first hour, 0.2 mg being infused during the second hour, and 0.1 mg being infused during the third hour. They reported: "The most striking finding in our study was that the 6 day urinary excretion of digoxin after a 3 hr infusion was 21% more than after the same dose of drug given during the 1 hr infusion."

Linear pharmacokinetic theory indicates that the total area under the serum or plasma concentration-time curve, AUC $0\text{-}\infty$, and the total amount excreted in the urine, Ae^{∞}, after single doses of a drug should be the same whether the drug is administered as a bolus injection, as a 1 hr infusion, or as a 3 hr infusion. This was the hypothesis tested by the study we report on herein.

EXPERIMENTAL

Human Study

Six young adult male volunteers with no known disease who weighed between 66 and 96 kg were selected. Normal complete physical examination, routine blood and urinalysis, normal values for kidney and liver function tests, and normal resting 12 lead electrocardiograms were necessary for entry into the study. Informed consent was obtained from each subject.

A recent drug history was taken for each prospective subject. All subjects participating in the study received no barbiturates or other enzyme-inducing agents for a period of 30 days preceding initiation of the study and

none concurrent with it. They received no other medication or alcoholic beverages for a period of 7 days before initiation of the study and none during the study.

At 8:30 p.m. each night before dosing with digoxin, the subjects were admitted to the Clinical Research Center of University Hospital, The University of Michigan. They ate a snack at 9:30 p.m. that night, then fasted from that time until 4 hr after dosing with digoxin the next day. On the days of dosing, they ate standard lunches and suppers, which combined provided 1240 calories consisting of 22% protein, 17% fat, and 61% carbohydrate. Subjects drank lemonade and received neither tea or coffee on the days of dosing. Subjects were supine during the days they received digoxin.

Treatments were as follows. A: 2cc of digoxin injection containing a label dose of 0.5 mg of digoxin was given. The solutions used for treatments B, C, and D were prepared under sterile conditions by adding the contents of the same lot of digoxin injection to sterile 5% dextrose in water. B: a label dose of 0.5 mg of digoxin in 240 ml of 5% dextrose was infused intravenously at a constant rate over a 1 hr period. C: a label dose of 0.5 mg digoxin in 360 ml of 5% dextrose was infused intravenously at a constant rate over a 3 hr period. D: a label dose of 0.5 mg digoxin in 240 ml of 5% dextrose was administered orally; then the container was rinsed and the contents swallowed.

The treatments were administered to the subjects as indicated by the study plan shown in Table I. There were three intravenous treatments; hence, there were six possible treatment sequences for these, and each subject received a different iv treatment sequence in crossover fashion. For the fourth phase, all received the oral treatment. Treatments were separated by two week periods.

Ten milliliters of whole blood were taken at zero time (just before dosing) and at 0.167, 0.333, 0.5, 0.75, 1, 1.5, 2, 3, 4, 8, 24, 48, 72, and 96 hr after treatment A; 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 9, 25, 49, 73, and 97 hr after treatment B; 1, 2, 3, 3.25, 3.5, 3.75, 4, 5, 6, 7, 11, 27, 51, 75, and 99 hr

Table I. Treatment Schedule in Human Study

	Treatment in indicated phase			
Subject	I	II	III	IV
1	A	B	C	D
2	B	C	A	D
3	C	A	B	D
4	A	C	B	D
5	B	A	C	D
6	C	B	A	D

after treatment C; and 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 24, 48, 72, and 96 hr after treatment D. Prior experience in performing digoxin bioavailability studies had indicated that these sampling times defined well the concentration-time curves after the different methods of administration. Blood was drawn in vacutainers containing sodium heparin. Plasma, obtained by centrifugation shortly after withdrawal of the blood, was quick-frozen and stored at −20°C until just before assay. Urine was collected in 12 hr periods for 6 days after dosing. After measurement of the volume and adequate mixing, a 30 ml aliquot of each urine was frozen and maintained at −20°C until just prior to assay.

Assay of Plasma and Urine Samples

Plasma and urine samples were assayed by the radioimmunoassay method of Wagner *et al.* (3). This assay measures digoxin down to 0.05 ng digoxin per ml of plasma. Each sample was assayed independently by two different analysts, and the results were averaged. Calibration data were obtained by each analyst on each day unknowns were assayed. For plasma and urine, these standards contained 0, 0.05, 0.1, 0.5, 1, 2, and 5 ng digoxin per milliliter. For all days, the 0.05 ng/ml plasma was distinguished from the 0 sample by having a lower percent bound value. The plasma assay utilized 0.5 ml of plasma, while the urine assay employed only 10 μl of urine and 100 μl of digoxin-free plasma. For calibration purposes, the normalized fraction of digoxin bound, F, is given by

$$F = B(x)/B(0) \tag{1}$$

where $B(x)$ is the percent digoxin bound at the concentration C and $B(0)$ is the percent digoxin bound in the absence of digoxin. Each day of assay, each analyst's binding data for the above standards gave a straight line by the method of least squares based on the logarithmic logistic equation

$$\ln[(1-F)/F] = \ln Q + S \ln C \tag{2}$$

where $\ln Q$ is the intercept (corresponding to $C = 1$), and S is the slope when the lefthand side of Eq. (2) was plotted against $\ln C$. Correlation coefficients for individual standard curves varied from 0.991 to 1.00 with the vast majority being 0.999. Concentrations of unknowns were inversely estimated by rearranging Eq. (2) to Eq. (3):

$$C = e^{\{\ln[1-F/F]-\ln Q\}/S} \tag{3}$$

On a given day, each analyst analyzed all the plasma samples or all the urine samples of one subject. The values of $\ln Q$ and S obtained from the

calibration standards run that same day by the same analyst were then substituted into Eq. (3) to calculate digoxin concentrations of the unknown samples. Errors involved in this assay have been discussed elsewhere (3). Samples containing >5 ng digoxin/ml were diluted.

Multiple radioimmunoassays were performed on the ampules of the same lot used for bolus injections and on the three solutions used for treatments B, C, and D to obtain the doses by assay, which were 447, 479, 492, and 547 μg for treatments A, B, C and D, respectively. The solution remaining in the infusion tubing after treatments B and C was blown out, the tubing was rinsed, and this mixture was assayed for each subject. Then this amount was subtracted from the 479 and 492 μg above to give the actual dose per subject. Usually such care is not taken, but the differences of label from actual doses are large enough that appreciable error would be introduced if they are not accounted for.

RESULTS AND EVALUATION

Table II lists the average digoxin plasma concentrations and the corresponding coefficients of variation. Table III lists the average urinary excretion rates and the average amounts excreted in the urine in 6 days as well as the mean 0 to ∞ values. Table IV gives the mean values and the coefficients of variation of pharmacokinetic parameters, which were estimated by the methods given in the Appendix.

Since the study design (Table I) involved a crossover design utilizing all six possible treatment sequences for the three methods of intravenous administration, an analysis of variance for crossover design was performed for each set of such data comprising 18 numbers. The underlined mean values in each row of Table IV indicate that the differences among the means were significant when analyzed by this method. The results of these analyses are summarized in Table V. The fourth column of Table V indicates that in no case was the *among periods* mean square significant. For several of the parameters, the oral data were then added to the intravenous data (total of 24 numbers per set), and a two factor analysis of variance was performed. Results are listed in Table VI.

Table VII lists the mean apparent systemic bioavailabilities estimated by three different methods involving different assumptions. The equations used are given as Eq. (A1) through (A17) in the Appendix. Results of two factor analyses of variance of these data are given in the last three rows of Table VI. It should be noted that since both total clearance and nonrenal clearance of digoxin changed significantly with the method of intravenous administration, none of the three methods of estimating systemic availability of digoxin are truly valid, but the means are given for comparison purposes.

Table II. Average Plasma Concentrations (C) of Digoxin with Their Coefficients of Variation (cv %)

Bolus iv			1 hr inf.			3 hr inf.			Oral solution		
Time (hr)	*C* (mg/ml)	CV (%)	Time (hr)	*C* (mg/ml)	CV (%)	Time (hr)	*C* (mg/ml)	CV (%)	Time (hr)	*C* (mg/ml)	CV (%)
0	0	0	0	0	0	0	0	0	0	0	0
0.167	25.9	63.6	0.25	4.21	23.5	1	2.21	40.9	0.25	0.52	80
0.333	9.68	70.7	0.5	5.88	15.2	2	2.87	26.4	0.5	1.83	48.7
0.5	5.52	26.7	0.75	6.80	20.0	3	2.63	45.7	0.75	2.38	24.0
0.75	3.77	21.8	1	6.36	18.5	3.25	1.84	42.8	1	2.10	21.3
1	2.84	22.8	1.25	3.47	19.7	3.5	1.45	41.2	2	1.03	27.0
1.5	1.79	20.2	1.5	2.53	22.5	3.75	1.19	41.0	3	0.60	27
2	1.30	21.2	1.75	1.93	25.1	4	0.98	42	4	0.54	32
3	0.96	27	2	1.57	28.3	5	0.60	40	6	0.365	47
4	0.75	18	3	0.86	26	6	0.49	30	8	0.28	41
8	0.45	26	4	0.69	17	7	0.45	30	24	0.175	57
24	0.25	26	5	0.49	17	11	0.34	34	48	0.12	73
48	0.14	33	9	0.40	13	27	0.23	24	72	0.056	94
72	0.088	42	25	0.22	24	51	0.13	52	96	0.031	94
96	0.052	54	49	0.14	27	75	0.0875	69			
			73	0.083	46	99	0.0375	73			
			97	0.058	61						

Table III. Average Urinary Excretion Rate and Amounts Excreted in Urine with Their Coefficients of Variation

	Urinary excretion rate (μg/day)							
	Bolus iv		1 hr inf.		3 hr inf.		Oral solution	
Day	Rate (μg/day)	CV (%)	Rate (μg/day)	CV (%)	Rate (μg/day)	CV (%)	Rate (μg/day)	CV (%)
1	182	15.1	134	43.6	159	11.9	94.2	31.5
2	49.2	20.6	42.0	22.6	55.6	56.0	46.0	46.0
3	34.1	19.2	29.4	28.1	37.5	43.2	28.1	28.5
4	17.9	45.2	17.9	34.2	16.1	36.2	14.1	28.8
5	10.5	28.0	8.29	32.5	8.64	57.5	8.02	58.7
6	8.48	71.5	7.86	50.0	6.23	43.9	5.17	39.4
0–6[a]	302	6.52	236	22.5	283	16.9	198	22.9
0-∞[b]	308	6.33	244	19.9	287	16.9	204	23.3

[a] Average amount of digoxin excreted in urine in 6 days (μg).
[b] Estimated average amount of digoxin excreted in urine in infinite time (μg).

Considerable effort was expended in estimating by different methods several of the pharmacokinetic parameters and comparing the results statistically to ensure that observed differences were not artifacts. Areas under the plasma concentration-time curves, AUC O-T, to the last sampling time T (96 hr postadministration for treatments A and D and 96 hr postinfusion for treatments B and C) were obtained by applying the trapezoidal rule up to the peak concentration, then the logarithmic trapezoidal rule from the peak concentration onward. Details are given in the Appendix. These areas were then corrected for the dose by assay symbolized by D. The means and coefficients of variations of these areas are given in the first row of Table IV. The estimated total areas under the concentration-time curves, AUC 0-∞, were estimated by three different methods as described in the Appendix. The mean dose-corrected total areas per 0.5 mg of digoxin are shown in the third row of Table IV, and these areas were calculated by application of Eq. (A3) of the Appendix. These areas, AUC 0-∞, estimated by different methods, agreed very well with each other. The mean of 24 areas (6 subjects × 4 treatments) obtained by application of Eq. (A1) through (A3) of the Appendix, namely, 25.61 (ng/ml) hr, did not differ significantly from the mean area of 25.76 (ng/ml) hr obtained by polyexponential fitting followed by integration, as indicated by Eq. (A7) through (A9) in the Appendix (paired $t = 0.40$, $p > 0.25$). Also, the mean area of 27.85 (ng/ml) hr, obtained by applying Eq. (A1) through (A3), did not differ significantly from the mean area of 28.29 (ng/ml) hr obtained by application of Eq. (A4) for 22 sets of data, where such a comparison was feasible (paired $t = 1.89$, $0.10 > p > 0.05$).

Table IV. Summary of Average Parameter Values and Coefficients of Variation

Parameter	Symbol and dimensions	Mean Parameter Value with Coefficient of Variation: Bolus iv	1 hr inf.	3 hr inf.	Oral solution
Dose-corrected area per 0.5 mg of digoxin	$\left[\frac{AUC}{0\text{-}T}\right]\left[\frac{0.5}{D}\right]\left[\frac{ng}{ml}\times hr\right]$	32.85[a] (14.8)[b]	26.85 (21.0)	23.75 (35.1)	14.50 (47.2)
Fraction of dose excreted in urine in 6 days	A_e^6/D	0.675 (6.53)	0.500 (22.9)	0.579 (17.0)	0.361 (23.0)
Dose-corrected total area per 0.5 mg digoxin	$\left[\frac{AUC}{0\text{-}\infty}\right]\left[\frac{0.5}{D}\right]\left[\frac{ng}{ml}\times hr\right]$	35.55 (14.8)	30.20 (27.7)	25.80 (35.5)	15.47 (49.9)
Fraction of dose excreted in urine from 0 to ∞	A_e^∞/D	0.689 (6.31)	0.517 (20.4)	0.588 (16.8)	0.374 (23.4)
Total clearance	Cl_t (ml/min)	239 (16.2)	300 (35.7)	366 (41.8)	675 (51.5)
Renal clearance	Cl_r (ml/min)	164 (10.8)	157 (49.4)	215 (42.3)	240 (50.3)
Nonrenal clearance	Cl_{nr} (ml/min)	75 (30.5)	143 (31.9)	151 (54.3)	435 (58.8)
Nonrenal clearance as fraction of total clearance	Cl_{nr}/Cl_t	0.309 (14.5)	0.483 (21.6)	0.413 (24.0)	0.627 (13.9)
Elimination rate constant	β (hr^{-1})	0.0252 (24.1)	0.0263 (33.1)	0.0313 (27.9)	0.0340 (25.1)
Elimination halflife	$0.693/\beta$ (hr)	28.6 (19.2)	28.2 (23.9)	23.5 (25.5)	21.6 (26.0)
Volume of distribution	V_β (liters)	580 (12.9)	690 (19.7)	716 (30.9)	[c]

[a] Mean parameter value of 6 subjects; see text for meaning of underlining.
[b] Bracketed number is the coefficient of variation in percent.
[c] Should not be calculated for oral administration.

It should be noted that the method of estimating the AUC's for the bolus intravenous treatment gave the most conservative values possible. The area under the initial part of the concentration-time curve was estimated as area of the triangle formed from the origin (0,0 point), the concentration at 0.167 hr, and at 0.167 hr. In estimating AUCs from polyexponential fitting of the bolus concentration-time data, the polyexponential equation was integrated from 0.167 hr to infinity, then the area of the triangle discussed above was added. If one used the C_0 value obtained by polyexponential fitting in estimating the area, much higher AUC values were obtained, and the discrepancy between AUCs following bolus injection and the infusions would have been greater.

Table V. Summary of Results of Analyses of Variance for Crossover Design (iv Data Only)

Parameter	Mean square or variance				Coefficient of Variation (%)	
	Among subjects	Among treatments	Among periods	Residual	Overall	From residual mean square
Dose-corrected area per 0.5 mg digoxin	90.4 ($0.05 > p > 0.025$)	128.3 ($0.025 > p > 0.01$)	2.56 NS($p > 0.25$)	20.9	25.9	19.0
Fraction of dose excreted in urine in 6 days	0.627×10^{-2} NS($p > 0.25$)	4.59×10^{-2} ($p = 0.025$)	1.59×10^{-2} NS($p > 0.10$)	0.758×10^{-2}	19.2	14.8
Dose-corrected total area per 0.5 mg digoxin	143.9 ($0.01 > p > 0.005$)	145.3 ($0.01 > p > 0.005$)	16.6 NS($p > 0.25$)	19.34	27.5	14.4
Fraction of dose excreted in urine from 0 to ∞	0.537×10^{-2} NS($p > 0.025$)	4.50×10^{-2} ($0.025 > p > 0.01$)	1.94×10^{-2} NS($p > 0.05$)	0.604×10^{-2}	18.3	13.0
Total clearance	2.68×10^{4} ($0.025 > p > 0.01$)	2.40×10^{4} ($0.05 > p > 0.025$)	0.663×10^{4} NS($p > 0.25$)	0.427×10^{4}	38.6	21.7
Renal clearance	7.77×10^{3} NS($p > 0.25$)	5.96×10^{3} NS($p > 0.10$)	5.83×10^{3} NS($p > 0.10$)	2.90×10^{3}	39.6	30.2
Nonrenal clearance	0.632×10^{4} ($0.025 > p > 0.01$)	1.03×10^{4} ($0.025 > p > 0.01$)	0.259×10^{4} NS($p > 0.10$)	0.122×10^{4}	51.1	28.4
Nonrenal clearance as fraction of total clearance	0.538×10^{3} NS($p > 0.25$)	46.1×10^{3} ($0.025 > p > 0.01$)	19.7×10^{3} NS($p > 0.05$)	0.594×10^{3}	27.5	19.2
Elimination rate	1.49×10^{-4} ($0.005 > p > 0.001$)	6.39×10^{-5} ($0.10 > p > 0.05$)	3.99×10^{-5} NS($p > 0.10$)	1.54×10^{-4}	28.8	14.2
Volume of distribution	3.75×10^{4} NS($p > 0.25$)	3.12×10^{4} NS($p > 0.25$)	1.74×10^{2} NS($p > 0.25$)	2.21×10^{4}	23.7	22.3

Table VI. Summary of Results of Two Factor Analyses of Variance (All Four Treatments)

Parameter	Mean square or variance			Coefficient of variation (%)	
	Among subjects	Among treatments	Residual	Overall	From residual mean square
Dose-corrected area per 0.5 mg of digoxin	107 ($0.01 > p > 0.005$)	352 ($p < 0.001$)	21.6	43.3	19.0
Fraction of dose excreted in urine in 6 days	0.113×10^{-2} NS($p > 0.25$)	10.6×10^{-2} ($p < 0.001$)	1.02×10^{-2}	27.4	19.1
Dose-corrected total area per 0.5 mg of digoxin	148.4 ($0.01 > p > 0.005$)	434.6 ($p < 0.001$)	30.9	39.0	20.8
Fraction of dose excreted in urine from 0 to ∞	0.049×10^{-2} NS($p > 0.25$)	10.5×10^{-2} ($p < 0.001$)	0.998×10^{-2}	26.3	18.4
Renal clearance	1.70×10^{4} ($0.025 > p > 0.01$)	9.62×10^{3} NS($p > 0.10$)	4.05×10^{3}	44.9	32.8
Elimination rate constant from plasma data (hr^{-1})	1.66×10^{-4} ($0.005 > p > 0.001$)	1.04×10^{-4} ($p = 0.05$)	3.19×10^{-5}	28.7	19.3
Elimination halflife (hr)	7.95×10 ($0.025 > p > 0.01$)	7.24×10 ($0.05 > p > 0.025$)	2.06×10	25.0	17.8
Apparent systemic availability: Method A	3.00×10^{-3} NS($p > 0.25$)	2.28×10^{-1} ($p < 0.001$)	1.17×10^{-2}	25.1	13.9
B	8.17×10^{-4} NS($p > 0.25$)	2.16×10^{-1} ($p < 0.001$)	1.96×10^{-2}	26.3	17.8
C	6.89×10^{-2} ($0.05 > p > 0.025$)	3.39×10^{-1} ($p < 0.001$	2.10×10^{-2}	36.0	19.3

Table VII. Apparent Systemic Availabilities

Method	Assumption	Average apparent systemic availabilities			
		Bolus iv	1 Hr Inf.	3 Hr inf.	Oral
A	Constant Cl_{nr} but variable Cl_r	1.00	0.772 (13.5)[a]	0.807 (10.1)	0.526 (27.2)
B	Proportional change in Cl_{nr} with change in Cl_r	1.00	0.753 (22.3)	0.853 (15.5)	0.584 (26.8)
C	Constant Cl_t	1.00	0.845 (23.2)	0.720 (30.0)	0.439 (49.2)

[a]Number in parentheses is coefficient of variation (%).

Apparent elimination rate constants, β, were estimated by three different methods: one using Eq. (A3), a second using Eq. (A5), and a third using Eq. (A3), except that $(Ae)_i$ and $(Ae)_{i+1}$ replaced $(AUC)_i$ and $(AUC)_{i+1}$, respectively. With three β values per subject per treatment (total 72 values), a three factor analysis of variance with subjects, treatments, methods, and residual as sources of variation was carried out. The mean square for methods, 0.44, was not significant ($p > 0.25$); the mean square for treatments, 3.25, was significant ($0.05 > p > 0.025$), as well as the mean square of 5.17 for subjects ($p < 0.001$). Thus with these data, there was evidence that the elimination rate constant increased with decrease in the rate of input of digoxin to the body (i.e., bolus iv > 1 hr inf. > 3 hr inf. > oral). The three estimates of β for each subject in each treatment were averaged, then these six values were averaged again to provide the mean β values shown in the ninth column of Table IV. However, when the 18 average β values for the three intravenous treatments were analyzed by analysis of variance for crossover design, or the 24 average β values for all four treatments were analyzed by two factor analyses of variance, there were no significant differences among the treatment averages (9th row of Tables IV and V, and 6th row of Table VI).

DISCUSSION

In our study, both the total and nonrenal clearances of digoxin differed significantly with the method of administration. The portal vein concentration-time profiles of digoxin following oral administration of digoxin extend beyond 6 hr (4), indicating that oral administration provides a slower input rate to the body than a 3 hr infusion. Thus in our study, the slower the rate of input of digoxin to the body, the greater were both the total clearance

and the nonrenal clearance. This strongly suggests nonlinear pharmacokinetics, and specifically, some type of saturation phenomenon in the liver. The results obtained in our study are the opposite of those reported by Greenblatt *et al.* (1). They reported that 6 day urinary excretion after rapid iv injection, 69% of the dose, was significantly ($p < 0.001$) lower than after 1 hr infusion, 76%, and variation of excretion was greater after rapid injection than after 1 hr infusion. Our data (Table IV) show that 6 day urinary excretion after rapid iv injection, 67.5% of the dose, was significantly greater than after 1 hr infusion, 50% of the dose, and the coefficient of variation, 6.53%, after rapid iv injection was much less than the corresponding value of 22.4% following 1 hr infusion. So far as ratios are concerned, results obtained in our study agreed with those reported by Marcus *et al.* (2). They reported that 60, 70, and 51% of the dose (ratios of 1.0, 1.17, and 0.85) were excreted in the urine in 6 days following 1 hr infusion, 3 hr infusion and oral solution, respectively; whereas in our study, the corresponding values (row 2 of Table IV) were 50, 58, and 36% of the dose (ratio 1.0, 1.16 and 0.72) for the same routes of administration, respectively. Unfortunately, clearances could not be estimated from either the data of Greenblatt *et al.* (1) or Marcus *et al.* (2) since they sampled blood only over 8 and 6 hr, respectively (5).

Another type of nonlinearity was reported by Huffman *et al.* (6). They reported that doubling of the digoxin dose given by bolus intravenous injection from 0.125 to 0.25 mg doubled the amount of digoxin excreted in the urine during a dosage interval at steady state, whereas the area under the serum digoxin concentration-time curve at steady state increased by only 50% for oral administration and only 80% for intravenous administration. In linear pharmacokinetics, the amount excreted during a dosage interval at steady state is equal to the amount excreted from zero to infinite time after a single dose. In the study of Huffman *et al.* (6), the amounts excreted during a dosage interval at steady state were measured. In our study and in the study of Koup *et al.* (7), Ae^{∞} was estimated. From these urinary excretion data reported in the three studies, one can calculate that the average fractions of the doses excreted in the urine were 0.401, 0.418, 0.675, and 0.755 for the 0.125, 0.25, 0.5, and 0.75 mg doses, respectively. Thus this interstudy comparison also indicates a type of nonlinearity in digoxin pharmacokinetics.

Some other comparisons of our results with those reported by others are noteworthy. Lloyd *et al.* (8) appropriately collected sufficient data and evaluated it correctly to allow estimation of total clearance of digoxin. From their data (8), one can calculate a mean total clearance of digoxin of 328 ml/min following administration of 0.4 mg doses infused intravenously over 1 hr, which agrees quite well with the mean total clearance of

300 ml/min (5th row of Table IV) obtained following the 1 hr infusion in our study. Lisalo (9), in a review, summarized urinary excretion of digoxin following single intravenous doses where urine was collected from 6 to 10 days; the percentages were 70.5, 70.1, 80, 76, 57, 68, and 70.8% (average 70%). Our mean percentage of 67.5% folowing the bolus intravenous method (row 2 of Table IV) agrees well. The apparent systemic availability of digoxin of 44 to 55% of the oral dose in solution form in our study (Table VII) is considerably lower than that reported by other authors, as summarized by Greenblatt *et al.* (10). In a previous study (11), Wagner *et al.* reported a systemic availability of 80% of the dose, based upon AUC 0–96 hr, when 0.5 mg doses of digoxin were administered orally in the same type of solution as used in the study being reported herein. However, reevaluation of those data by Wagner and Ayres (5) and estimation of AUC $0\text{-}\infty$ indicated that the digoxin was completely absorbed (100%) after the oral solution rather than the originally reported 80%.

One possible variable that could have affected the results in our study was posture. In the present study, the subjects were supine (horizontal) during most of the day when digoxin was administered, whereas in previous digoxin bioavailability studies performed by the senior author, subjects were always ambulatory (upright). Culbertson *et al.* (12) reported that estimated hepatic blood flow, as measured by intravenous bromsulfalein, decreased in normotensive subjects by 37.5% (from a mean of 1.71 liters/min/1.73 m^2 to 1.07 liters/min/1.73 m^2) when subjects changed from the horizontal to the upright body position. Smith and Shimizo (13) reported that lithium renal clearance was 30% lower while standing than while reclining; also, clearances of creatinine, sodium, and potassium were all lower while standing than while reclining. The only other digoxin bioavailability study that was performed when the normal volunteers were supine that we could find in the literature was that of Huffman and Azarnoff (14). Their data differed from most subsequent digoxin bioavailability data in that following intravenous and oral administration of 0.5 mg doses, only 57 and 53% of the doses, respectively, were excreted in the urine in 10 days. Thus although these percentages are similar, the percentage of the dose excreted was low relative to most other reported data. It is feasible that in the supine posture and oral administration, a greater portion of the digoxin dose gets metabolized in the liver as a result of higher effective liver blood flow, and, also, that less gets absorbed as a result of less physical activity; hence, less motility and/or contact of part of the digoxin solution in the lumen of the gastrointestinal tract with the absorbing membrane. One final comparison is of interest. In our study, the overall mean renal clearance of digoxin was 194 ml/min (average of the four values in row 6 of Table IV), which agrees very well with the average of 191 ml/min reported by Keller *et al.* (15).

It is unlikely that the results we report were caused by the nonspecificity of the digoxin radioimmunoassay. Seventeen randomly selected plasma samples from this study were assayed not only by the direct RIA (nonspecific method), but also by a method in which digoxin was separated from its metabolites by HPLC, the digoxin fraction collected at the end of the HPLC column, then the digoxin assayed by RIA (specific method). The mean nonspecific assay, 1.92 ng/ml, did not differ significantly from the mean specific assay, 1.79 ng/ml (paired $t = 1.21, p > 0.10$). Similarly, 34 randomly selected 24-hr urine collections from days 1 through 4 were assayed by the two methods. The mean nonspecific assay, 15.5 ng/ml, did not differ significantly from the mean specific assay, 15.4 ng/ml (paired $t = 0.12$, $p > 0.25$). Details of our specific digoxin assay will be published elsewhere. Relatively low concentrations of digoxin metabolites in both plasma or serum and urine, capable of cross-reacting in the radioimmunoassay, have been reported for subjects and patients with normal renal function recently by others (16,17). Gault *et al.* (16) administered 150 μCi ^{3}H-digoxin-12α orally to six subjects with normal renal function and collected urine for 5 days. Digoxin and its metabolites were separated using diethylaminoethyl Sephadex LH-20 column chromatography, and the amount of each was measured. Mean cumulative percentages of the ingested radioactivity excreted in the 5 days in the urine were 54.5% digoxin, 0.5% bis-digitoxoside, 0.19% monodigitoxoside, 0.03% digoxigenin, and 0.03% dihydrodigoxin. Gibson and Nelson (17) administered digoxin to nine subjects with various degrees of renal function but not requiring dialysis, collected plasma samples, separated digoxin from its metabolites by high performance liquid chromatography, then collected the fraction corresponding to digoxin and measured the digoxin by radioimmunoassay. They also assayed the sera using the RIA directly. The ratio of HPLC digoxin/direct RIA digoxin averaged 1.06 with a range of 0.94 to 1.24. Thus the percentages of digoxin metabolites, relative to digoxin itself in both plasma and urine of volunteers with normal renal function, are very small. Reports of metabolites of digoxin in patients with impaired renal function and/or cardiac disease are not pertinent to this report involving normal volunteers.

There is another point of interest in the urinary data reported by Gault *et al.* (16). Their semilogarithmic plots of urinary excretion rates versus time show that each metabolite has a markedly different elimination rate constant and halflife than that of digoxin. If urinary excretion rate constants of the metabolites are greater than their formation rate constants, one would expect at least the corresponding bis-glycoside and dihydrodigoxin to have the same elimination halflives as digoxin as a result of the precursor-product relationship in a catenary chain with parallel paths. The fact that they don't suggests that all of the metabolites may have been formed on the "first pass" through the liver following oral administration.

A similar finding, as we report here for digoxin in man, was reported by Frey *et al.* for prednisolone in the dog (18). Using the total clearance estimated from bolus iv data resulted in overestimation of the steady state concentration following infusions. Thus the total clearance for infusions was greater than the total clearance obtained from bolus iv data.

It should be noted that the mean areas under the plasma concentration-time curves from 0 to 8 hr after bolus intravenous and from the start of the infusions to 8 hr after the infusions ceased for the 1 and 3 hr infusions in our study did not differ significantly ($F = 2.83$, $0.25 > p > 0.10$) when analyzed by analysis of variance for crossover design. However, the mean dose corrected areas per 0.5 mg of digoxin (AUC 0–96 hr) and mean dose corrected total areas per 0.5 mg of digoxin (AUC 0-∞) were highly significantly different in our study (see Table V, rows 1 and 3). These data support the conclusions and simulations done by Wagner and Ayres (5), and indicate that much of the plasma or serum digoxin concentration-time data in the literature is of little use, since samples were not taken for a sufficiently long period of time, and no extrapolation to infinite time was possible. These data also indicate that the suggested time of blood sampling of 6 hr recommended by the Food and Drug Administration is a poor choice.

Since in our study, both the total clearance and the nonrenal clearance of digoxin changed significantly with the method of intravenous administration, there are no suitable equations to estimate digoxin bioavailability. Also, since in our study an infinitely dilutable aqueous solution of digoxin without digoxin precipitation (as can occur with the injectable form or as alcohol elixir) gave very low amounts in the urine and relatively low areas, this brings up many questions about the assessment of digoxin bioavailability as it has been done in the past.

APPENDIX

Methods of Estimating Areas Under Plasma Concentration-Time Curves

In general, the area under the concentration-time curve, $(AUC)_i$, to time t_i was estimated by means of Eq. (A1):

$$(AUC)_i = \int_0^{t_p} C\,dt + \int_{t_p}^{t_i} C\,dt \qquad \text{(A1)}$$

where t_p is the time of the observed peak concentration (10 min or 0.167 hr for bolus iv, 1 hr for the 1 hr infusion, 3 hr for the 3 hr infusion, and 0.75, 0.75, 1, 0.75, 0.5, and 0.75 hr for subjects 1 through 6, respectively, after oral administration). The first integral on the righthand side of Eq. (A1) was estimated by trapezoidal rule, while the second integral on the righthand side was estimated by means of the logarithmic trapezoidal rule. For bolus iv

administration, the area estimated was the most conservative one, since the first integral was obtained by assuming zero concentration at zero time and, hence, the first integral on the righthand side of Eq. (A1) was estimated using Eq. (A2):

$$\int_0^{t_p} C\,dt = \tfrac{1}{2}(0.167)(C_{0.167}) \tag{A2}$$

where $C_{0.167}$ was the observed plasma concentration at 0.167 hr. When $t_i = T = 96$ hr, then $(\text{AUC})_i = \text{AUC } 0\text{-}T$; the mean areas per 0.5 mg dose of digoxin obtained in this manner are shown in row 1 of Table III. Total areas, AUC 0-∞, were estimated by three different methods.

Method 1

This utilized the method of Wagner and Ayres (5), and is based on Eq. (A3), initially published by Wagner *et al.* (19):

$$(\text{AUC})_i = \text{AUC } 0\text{-}\infty - \left[\frac{1}{1-e^{-\beta\Delta t}}\right][(\text{AUC})_{i+1} - (\text{AUC})_i] \tag{A3}$$

In applying this method to concentrations measured following bolus iv and oral administrations, the $(\text{AUC})_i$'s were AUC 0–24, AUC 0–48, AUC 0–72, and AUC 0–96; for the 1 hr infusion data, they were AUC 0–25, AUC 0–49, AUC 0–73, and AUC 0–97; and for the 3 hr infusion data, they were AUC 0–27, AUC 0–51, AUC 0–75, and AUC 0–99. The method of least squares was applied to the four areas for a given subject after a given treatment using the $(\text{AUC})_i$'s as ordinate (y) values and the differences $(\text{AUC})_{i+1} - (\text{AUC})_i$ as the abscissa (x) values. The intercept was the desired total area, AUC 0-∞, and from the slope the elimination rate constant, β, was obtained with $\Delta t = 24$ hr.

Method 2

This was based on the classical method shown in Eq. (A4):

$$\text{AUC } 0\text{-}\infty = \text{AUC } 0\text{–}96 + \hat{C}_{96/\beta} \tag{A4}$$

Here AUC 0–96 was obtained using Eq. (A1) above. The second term on the righthand side of Eq. (A4) is an estimate of the area from 96 hr to infinity. In this method, the elimination rate constant was estimated by applying the method of least squares to plasma concentrations, C, measured at the last four sampling times using Eq. (A5),

$$\ln C = \ln B - \beta t \tag{A5}$$

then obtaining the estimated concentration at 96 hr, symbolized by $\hat{C}_{96}$ using Eq. (A6);

$$\hat{C}_{96} = e^{[\ln B - 96\beta]} \tag{A6}$$

Method 3

This method was based on fitting a polyexponential equation to the concentration-time data, then integrating the equation to obtain the estimated total area. The number of exponential terms needed to obtain an excellent fit was either 3 or 4. For bolus iv, all data of each set (except a 0,0 point) were fitted to Eq. (A7),

$$C = \sum_{i=1}^{n} C_i e^{-\lambda i t} \tag{A7}$$

then the area from 0.167 hr to infinity was obtained using Eq. (A8):

$$\int_{0.167}^{\infty} C\,dt = \frac{Ci}{\lambda i} e^{-0.167\lambda i} \tag{A8}$$

To that area was added the area from 0,0 to 0.167 hr obtained with Eq. (A2) provide the desired AUC 0-∞. Oral data were fitted with Eq. (A7), then integrated from 0 to ∞ as shown by Eq. (A9):

$$\int_{0}^{\infty} C\,dt = Ci/\lambda i \tag{A9}$$

Postinfusion data were fitted to a polyexponential equation and that portion integrated to provide the area from the end of the infusion to infinite time. To this was added the area from zero to the end of the infusion, which was estimated by trapezoidal rule.

Method of Estimating the Amount of Digoxin Excreted in the Urine in Infinite Time

The method of least squares was applied using the amounts excreted from days 2 through 6 or 3 through 6 $(Ae)_i$ and a similar equation to Eq. (A3), except $(Ae)_i$ replaced $(\text{AUC})_i$, Ae^{∞} replaced AUC 0-∞, and $(Ae)_{i+1}$ replaced $(\text{AUC})_{i+1}$.

Methods of Estimation of Other Pharmacokinetic Parameters

Total clearance, Cl_t, was estimated with Eq. (A10):

$$Cl_t = D/(\text{AUC } 0\text{-}\infty) \tag{A10}$$

Renal clearance, Cl_r, was estimated with Eq. (A11):

$$Cl_r = Ae^{\infty}/(\text{AUC } 0\text{-}\infty) \tag{A11}$$

Nonrenal clearance, Cl_{nr}, was estimated with Eq. (A12):

$$Cl_{nr} = Cl_t - Cl_r \tag{A12}$$

Elimination rate constants, β, were estimated three different ways: (a) by use of Eq. (A3); (b) by use of 2 or 3 through 6 day cumulative urinary excretion and a modification of Eq. (A3), where $(Ae)_i$ and $(Ae)_{i+1}$ replace $(\text{AUC})_i$ and $(\text{AUC})_{i+1}$; and (c) by use of Eq. (A5) with the concentrations corresponding to the last four sampling times.
Elimination halflife, $t_{\frac{1}{2}}$, was obtained with Eq. (A13):

$$t_{\frac{1}{2}} = 0.693/\beta \tag{A13}$$

Volume of distribution, V_β, was obtained using Eq. (A14):

$$V_\beta = Cl_t/\beta \tag{A14}$$

Apparent systemic availability was calculated by three methods as follows.

Method A

This method assumes constant Cl_{nr} but variable Cl_r and is estimated by means of equation (A15) as reported formerly (19):

$$Fx/Fs = \frac{Ds(\text{AUC } 0\text{-}\infty)x}{Dx(\text{AUC } 0\text{-}\infty)s} - \frac{[Cl_r^s - Cl_r^x](\text{AUC } 0\text{-}\infty)x}{Dx} \tag{A15}$$

where x refers to other treatment (1 hr infusion, 3 hr infusion, or oral) and s refers to bolus iv. Equations reported by Kwan and Till (20) and Øie and Jung (21) are exactly equivalent to Eq. (A15).

Method B

This method assumes a proportional change in Cl_{nr} with change in Cl_r and was estimated by Eq. (A16):

$$Fx/Fs = \frac{(Ae^{\infty})x/Dx}{(Ae_{\infty})s/Ds} \tag{A16}$$

Method C

This method assumes constant total clearance and was estimated by Eq. (A17):

$$Fx/Fs = \frac{(\text{AUC})x\ Ds}{(\text{AUC})s\ Dx} \tag{A17}$$

ACKNOWLEDGMENT

We thank the nurses of the Clinical Research Center for their assistance.

REFERENCES

1. D. J. Greenblatt, D. W. Duhme, J. Koch-Weser, and T. W. Smith. Intravenous digoxin as a bioavailability standard: slow infusion and rapid injection. *Clin. Pharmacol. Ther.* **15**:510–513 (1974).
2. F. I. Marcus, J. Dickerson, S. Pippin, M. Stafford, and R. Bressler. Digoxin-bioavailability: formulations and rates of infusions. *Clin. Pharmacol. Ther.* **20**:253–259 (1976).
3. J. G. Wagner, M. R. Hallmark, E. Sakmar, and J. W. Ayres. Sensitive radioimmunoassay for digoxin in plasma and urine. *Steroids* **29**:787–807 (1977).
4. K.-E. Andersson, L. Nyberg, H. Dencker, and J. Göthlin. Absorption of digoxin in man after oral and intrasigmoid administration studied by portal vein catheterization. *Eur. J. Clin. Pharmacol.* **9**:39–47 (1975).
5. J. G. Wagner and J. W. Ayres. Bioavailability assessment: methods to estimate total area (AUC 0-∞) and total amount excreted (Ae^{∞}) and importance of blood and urine sampling scheme with application to digoxin. *J. Pharmacokin. Biopharm.* **5**:533-557 (1977).
6. D. H. Huffman, C. V. Manion, and D. L. Azarnoff. Absorption of digoxin from different oral preparations in normal subjects during steady state. *Clin. Pharmacol. Ther.* **16**:310–317 (1974).
7. J. R. Koup, D. J. Greenblatt, W. J. Jusko, T. W. Smith, and J. Koch-Weser. Pharmacokinetics of digoxin in normal subjects after intravenous bolus and infusion doses. *J. Pharmacokin. Biopharm.* **3**:181–192 (1975).
8. B. L. Lloyd, D. J. Greenblatt, M. D. Allen, J. S. Hermatz, and T. W. Smith. Pharmacokinetics and bioavailability of digoxin capsules, solution and tablets after single and multiple doses. *Am. J. Cardiol.* **42**:129–136 (1978).
9. E. Lisalo. Clinical pharmacokinetics of digoxin. *Clin. Pharmacokin.* **2**:1–16 (1977).
10. D. J. Greenblatt, T. W. Smith, and J. Koch-Weser. Bioavailability of drugs: the digoxin dilemma. *Clin. Pharmacokin.* **1**:36–51 (1976).
11. J. G. Wagner, M. Christensen, E. Sakmar, D. Blair, J. D. Yates, P. W. Willis, A. J. Sedman, and R. G. Stoll. Equivalence lack in digoxin plasma levels. *J. Am. Med. Assoc.* **224**:199–204 (1973).
12. J. W. Culbertson, R. W. Wilkins, F. J. Ingelfinger, and S. E. Bradley. The effect of the upright posture upon hepatic blood flow in normotensive and hypertensive subjects. *J. Clin. Invest.* **30**:305–311 (1951).
13. D.F. Smith and M. Shimizu. Effect of posture on renal lithium clearance. *Clin. Sci. Mol. Med.* **51**:103–105 (1976).
14. D. H. Huffman and D. L. Azarnoff. Absorption of orally given digoxin preparations. *J. Am. Med. Assoc.* **222**:957–960 (1972).
15. F. Keller, H. P. Blumehthal, K. Maertin, and N. Rietbrock. Overall pharmacokinetics during prolonged treatment of healthy volunteers with digoxin and β-methyldigoxin. *Eur. J. Clin. Pharmacol.* **12**:387–392 (1977).
16. M. H. Gault, D. Sugden, C. Maloney, M. Ahmed, and M. Tweeddale. Biotransformation and elimination of digoxin with normal and minimal renal function. *Clin. Pharmacol. Ther.* **25**:499–513 (1979).
17. T. P. Gibson and H. A. Nelson. The question of cumulation of digoxin metabolites in renal failure. *Clin. Pharmacol. Ther.* **27**:219–223 (1980).
18. F. J. Frey, B. M. Frey, A. Greither, and L. Z. Benet. Inequality of prednisolone clearance values obtained by iv bolus and by steady-state infusion. *Clin. Res.* **28**(2): 236A (1980).

19. J. G. Wagner, R. G. Stoll, D. J. Weidler, J. W. Ayres, M. R. Hallmark, E. Sakmar, and A. Yacobi. Comparison of the *in vitro* and *in vivo* release of digoxin from four different soft gelatin capsule formulations. *J. Pharmacokin. Biopharm.* **7:**147–158 (1979).
20. K. C. Kwan and A. E. Till. Novel method for bioavailability assessment. J. Pharm. Sci. **62:**1494–1497 (1973).
21. S. Øie and D. Jung. Bioavailability under variable renal clearance conditions. *J. Pharm. Sci.* **68:**128–129 (1979).