Journal of Pharmacokinetics and Biopharmaceutics, Vol. 7, No. 1, 1979

Pharmacokinetics of Digoxin: Relationship Between Response Intensity and Predicted Compartmental Drug Levels in Man

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Received June 7, 1977—Final May 12, 1978

A study designed to investigate the relationship between the pharmacokinetics of digoxin and a measure of its pharmacological effect has been conducted. Serum digoxin concentrations and systolic time intervals were measured concurrently in 12 normal male volunteers following a 1.0 mg i.v. bolus injection. The averaged serum digoxin concentration–time and response–time data were analyzed pharmacokinetically using a three-compartment open model and nonlinear least-squares fitting. When only the serum level–time data were analyzed, a close relationship was found between calculated digoxin levels in the slowly distributing (deep) peripheral compartment and response of the heart to digoxin, as measured by changes in the QS_2 *index* (ΔQS_2I). *Although it was not possible to distinguish clearly a linear from a nonlinear relationship between digoxin levels in the deep compartment and* ΔQS_2I, *the nonlinear relationship gave the best overall fit when both serum digoxin and* ΔQS_2I *data were fitted simultaneously. The simultaneous fit yielded a total body clearance of digoxin of* 3.6 *ml/min/kg and a terminal* $t_{1/2}$ *of 42 hr.*

KEY WORDS: digoxin; pharmacokinetics; response kinetics; three-compartment model; serum digoxin kinetics; systolic time intervals; radioimmunoassay.

Supported in part by Philips Roxane Laboratories, Columbus, Ohio, Medicinal Chemistry Training Grant GM-1949 (NIH) for W.G.K, Central Ohio Heart Chapter of AHA and National Heart and Lung Institute Training Grant No. 5-T01-HL05968 for A.J.K., and Clinical Research Center Grant RR-34 (PHS). This article is abstracted in part from a dissertation submitted by W.G.K. to the Graduate School, The Ohio State University, Columbus, Ohio, in partial fulfillment of the Doctor of Philosophy degree requirements.

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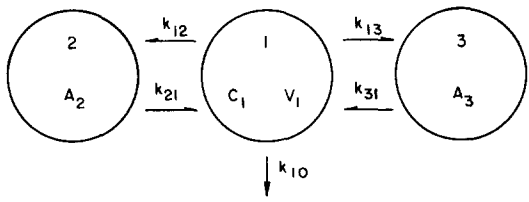
0090-466X/79/0200-0047$03.00/0

INTRODUCTION

Establishment of empirical relationships between the magnitude of pharmacological response and predicted compartmental drug concentrations in man provides a better understanding of the pharmacodynamics of drug action. Lewis *et al.* (1) have found that response of the heart to digoxin, as measured by changes in the electromechanical systole corrected for heart rate (QS_2I), is inversely proportional to a direct, invasive quantitation of inotropy. This correlation for the normal human ventricle occurs despite a small increase in peripheral resistance also thought to occur with digitalis (1,2) and demonstrates the usefulness of changes in $QS_2I(\Delta QS_2I)$ as a quantitative, noninvasive measure of the inotropic effect of digoxin in man. Lewis *et al.* (3) have recently reviewed the systolic time intervals (including QS_2I) with respect to correction factors for heart rate and diurnal variation, standards for equipment and technique, efforts at validation, and principles for assessing left ventricular performance. The serum level kinetics of i.v. digoxin have also been studied previously by Kramer *et al.* (4) and are consistent with a three-compartment open pharmacokinetic model. Thus both response and serum levels have previously been studied separately for digoxin in a quantitative manner. This article reports the concurrent measurement of changes in $QS_2I(\Delta QS_2I)$ and serum drug levels as a function of time after an intravenous dose of digoxin. The ΔQS_2I measurements are then correlated with calculated digoxin levels in the various compartments of the pharmacokinetic model.

THEORETICAL

The three-compartment open model used in this analysis is shown in Scheme I. Compartment 1 represents the central compartment, compartments 2 and 3 the peripheral or "tissue" compartments, C_1 the concentration of drug in and V_1 the apparent volume of compartment 1, A_2 and A_3 the amount of drug in compartments 2 and 3, and k_{ij} the apparent first-order rate constant for transfer of drug from the ith to the jth compartment ($j = 0$ represents an elimination process). This particular three-compartment open



Scheme 1

model was chosen because evidence suggests that digoxin is eliminated to a large extent by glomerular filtration, a process considered to originate from the central compartment (4).

The time course of drug levels in compartments 1, 2, and 3 following an i.v. bolus dose is described by equations 1–3 (5)

$$C_1 = (\text{dose}/V_1)(A\,e^{-\alpha t} + B\,e^{-\beta t} + C\,e^{-\gamma t}) \tag{1}$$

$$A_2 = A'\,e^{-\alpha t} + B'\,e^{-\beta t} + C'\,e^{-\gamma t} \tag{2}$$

$$A_3 = A''\,e^{-\alpha t} + B''\,e^{-\beta t} + C''\,e^{-\gamma t} \tag{3}$$

where A, B, C, A', B', C', A'', B'', C'', α, β and γ are complex functions of the k_{ij}'s of the model (see Appendix), "dose" is the administered dose, and t is time after injection.

In looking for relationships between pharmacological effect and predicted drug levels, the following procedure may be tried. First, the assumption can be made, as illustrated by Galeazzi *et al.* (6), that response is not necessarily related to levels in any compartment. This possibility can be examined by construction of plots of response vs. predicted level (see Appendix) for each compartment of the model. If an apparent relationship is found between response and level in a particular compartment, one can then proceed with the alternate hypothesis that

$$R = f(A_i) \tag{4}$$

where R is response and A_i the amount of drug in the ith compartment at the time the response is measured. Using nonlinear least-squares methodology, one can then fit the model to the response and serum concentration data simultaneously using an appropriate function to relate response to amount.

EXPERIMENTAL

Digoxin (Lanoxin Injection, Burroughs Wellcome, lot No. 644G) was administered to 12 healthy male volunteers by rapid i.v. injection (injection time <30 sec) at a dose of 1.0 mg. Each subject was given a physical examination with appropriate laboratory tests prior to entering the study. Blood chemistry and hematological values were within normal limits. Subjects were informed of the nature and hazards of the study and gave written consent. Subjects were kept nonambulatory and in a fasting state for 12 hr prior to and 4 hr after drug administration.

A 10-ml blood sample was drawn before drug administration to provide a blank for the assay. Blood samples (5 ml) were withdrawn from a forearm vein at 2, 4, 6, 8, 10, 14, 18, 22, 30, 45, and 60 min and 2, 3, 4, 5, 8, 12, 16,

24, 36, 48, 72, and 96 hr after drug injection. Samples taken during the first 4 hr were withdrawn through a 19-gauge infusion set kept open with a normal saline drip; later, samples were withdrawn by venipuncture. All samples were centrifuged, and the serum was separated and kept frozen until assay.

Systolic time intervals (STI) were obtained from simultaneous tracings of the electrocardiogram, phonocardiogram, and carotid arterial pulsations recorded on an Electronics for Medicine recorder at a paper speed of 100 mm/sec. Tracings were obtained before (control) and at 10, 20, 30, 45, 60, and 90 min and 2, 3, 4, 8, 12, 24, 48, 72, and 96 hr after drug administration. Subjects were kept hospitalized for 12 hr before and 24 hr after drug administration in order to minimize the effect of other factors, such as physiological changes due to a normal daily routine, on the STI measurements. Moderate activity, limited to the nursing unit, was permitted after the first 4 hr after dosing; however, each subject was required to rest for a 30–45 min period prior to each subsequent recording. Recordings taken following discharge from the hospital were also preceded by a rest period. The electromechanical systole corrected for heart rate (QS_2I) was calculated from the tracings by accepted techniques (1). Changes in QS_2I(ΔQS_2I) were determined as the difference between control and experimental values. Previous studies in our laboratories have demonstrated that a correction of ΔQS_2I is needed at certain times of the day in order to minimize the contribution of diurnal variation to the ΔQS_2I measurement (1). Mean values of the $|\Delta QS_2I|$ obtained in another group of untreated normal volunteers obtained over the same time cycle were used to correct the measured $|\Delta QS_2I|$ values in this study. Mean corrections were -3.4 msec ($N = 20$, SD $= 5.5$) at 4 hr, -10.1 msec ($N = 20$, SD $= 6.4$) at 8 hr, and -14.9 msec ($N = 15$, SD $= 10.4$) at 12 hr.

Serum samples were assayed using an ^{125}I-radioimmunoassay (Schwarz/Mann, catalogue No. 0750-06) modified slightly from the kit instructions to increase accuracy and precision. An additional standard was added at 0.2 ng/ml to establish the reproducibility of the assay at this level, incubation times were refined to maximize the antigen-antibody binding, and all microliter volumes were measured with Hamilton microliter syringes. All samples for a given subject were assayed in a single assay run that included a standard curve prepared from the subject's blank (zero-time) serum sample. By using each subject's blank serum, collected and stored under the same conditions as the experimental samples, effects on the assay due to subject-to-subject variability in serum proteins (7) or in other unknown factors are minimized. All samples were assayed in duplicate and the average value used.

Serum digoxin concentrations and ΔQS_2I's for each subject were determined and plotted against time. A certain amount of apparently

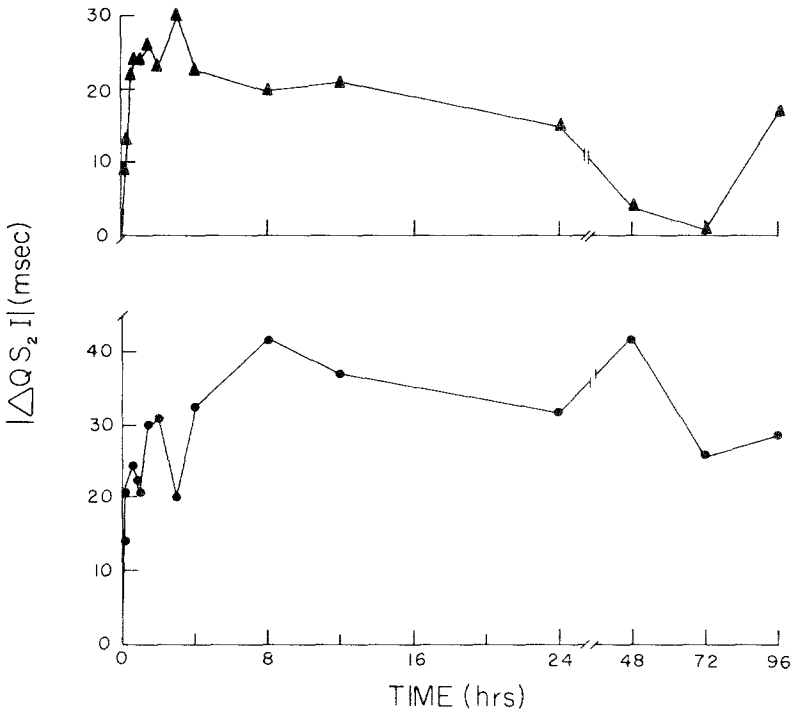


Fig. 1. Plot of $|\Delta QS_2I|$–time data for two individual subjects. ●, Subject S. R.; ▲, subject W. W.

random error, possibly due to changes in catecholamine levels brought on by the stress of the experimental situation, was evident on examination of the ΔQS_2I's of the individual subjects (Fig. 1). This apparent random error was minimized by averaging the 12 subjects' data, and all subsequent analyses were done with the averaged serum concentration–time and ΔQS_2I–time data sets.

Equation 1 was fitted to the averaged serum digoxin concentration–time data by weighted nonlinear least-squares regression analysis (8). Each data point was weighted by the reciprocal of its variance, and the five microconstants (k_{ij}'s) and V_1 were iterated to obtain the best fit according to the method of Kramer *et al.* (4). Initial estimates of the model parameters were obtained by graphical analysis of the experimental data. The resultant least-squares estimates of the microconstants of the model were then used in equations 1–3 to calculate either the serum digoxin concentration or the percent of the dose present in each compartment at each time that a STI had been recorded.

RESULTS

The averaged serum digoxin concentration–time data are listed in Table I. The same data are plotted in Fig. 2 together with the curve predicted for the central compartment from the nonlinear least-squares regression analysis of the serum concentration–time data only. The good agreement between experimental serum digoxin concentrations and the serum drug levels predicted by fitting with a three-compartment model is consistent with the results reported previously by Kramer *et al.* (4). The averaged ΔQS_2I data listed in Table I were used to construct plots of response vs. predicted digoxin level for each compartment of the model shown in Scheme I. The simplest relationship observed was for the ΔQS_2I data plotted as a function of the digoxin level in the most slowly distributing (deep) compartment, compartment 2 (Fig. 3). In Fig. 3 the predicted levels were obtained using

Table I. Averaged Serum Digoxin Concentration–Time and $|\Delta QS_2I|$–Time Data[a]

Time (min)	Serum digoxin concentration (ng/ml)	$\lvert\Delta QS_2I\rvert$ (msec)
2	63.8 (30.0)	—
4	35.5 (16.0)	—
6	30.2 (26.7)	—
8	21.0 (9.33)	—
10	16.4 (7.95)	14.0 (5.7)
14	17.3 (6.06)	—
18	13.9 (5.65)	—
20	—	15.9 (6.1)
22	11.4 (4.57)	—
30	8.57 (2.21)	18.8 (6.7)
45	7.06 (1.99)	22.7 (8.9)
60	5.74 (1.99)	24.6 (8.9)
90	—	23.4 (8.5)
120	2.79 (0.76)	24.6 (8.7)
180	1.95 (0.73)	27.9 (9.2)
240	1.64 (0.59)	24.4 (7.4)[b]
300	1.07 (0.36)	—
480	0.86 (0.37)	26.7 (12.8)[b]
720	0.60 (0.23)	22.6 (22.4)[b]
960	0.59 (0.16)	—
1440	0.58 (0.16)	20.6 (12.)
2160	0.38 (0.17)	—
2880	0.29 (0.12)	21.8 (14.)
4320	0.23 (0.12)	16.8 (12.)
5760	0.22 (0.20)	11.9 (13.)

[a] Numbers in parentheses are standard deviations. Twelve subjects participated in the study.
[b] Corrected for diurnal variation.

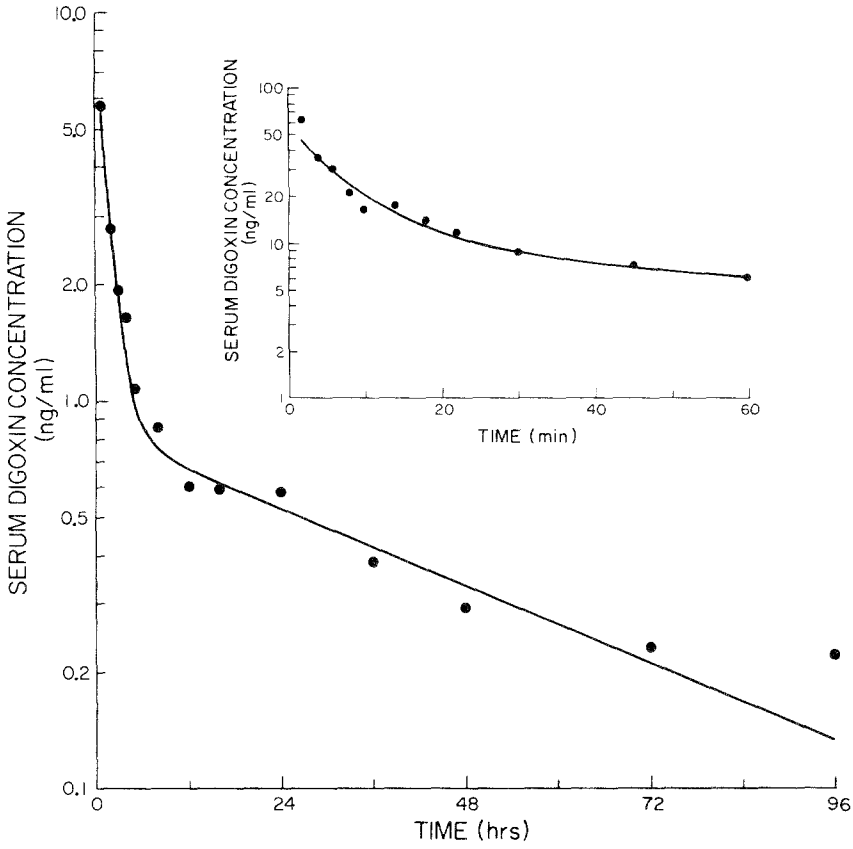


Fig. 2. Semilogarithmic plot of the averaged serum digoxin concentration–time data (●) and the curve (——) predicted by the nonlinear fitting of equation 1 to the data. Inset is an expanded time scale of the first hour.

equation 2 together with the microconstants from the fit of serum levels only.

The relationship shown in Fig. 3 may be viewed as either linear or nonlinear because the necessarily narrow range of ΔQS_2I measurements prevents discrimination between the two. If one adopts the linear hypothesis, the slope obtained by linear least-squares regression is 0.271 msec/% dose, the intercept is 10.9 msec, and the correlation coefficient is 0.91. In addition, points on the ascending and descending portions of the ΔQS_2I–time plot would be randomly distributed if such a regression line were drawn (Fig. 3). However, the finding that the y intercept for the linear relationship is significantly greater than 0 ($p < 0.05$) is inconsistent with the initial condition that there should be no response at

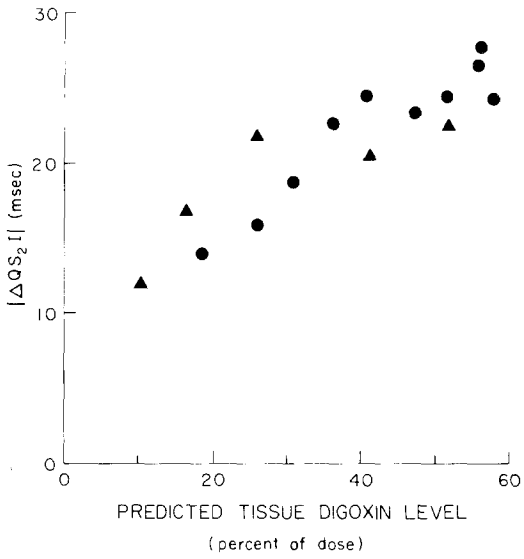


Fig. 3. Plot of the averaged $|\Delta QS_2I|$ data vs. the tissue digoxin level predicted for compartment 2 from the fit to the serum concentration–time data only. Points on the onset portion of the ΔQS_2I–time curve are shown as ● and those on the decay portion as ▲.

zero drug level. Also, the linear relationship does not accommodate the concept of a maximum response. Use of one of several available nonlinear relationships can circumvent these limitations. Wagner (9) has reviewed the various nonlinear relationships that have been used to fit response–concentration–time data. We have chosen one of the simplest of these, the Langmuir-type equation, since the limited range of the response measurements would preclude any differentiation between the various nonlinear relationships that are available (9). Thus, equation 4 may be replaced by

$$R = R_{\max} KA_2/(1+KA_2) \tag{5}$$

where $R_{\max}$ is the maximal response and K is a constant.

The results obtained by simultaneously fitting equations 1 and 5 to the serum digoxin concentration–time data and the ΔQS_2I–time data are shown in Figs. 4–6. Initial estimates for the K_{ij}'s and the value for V_1 were obtained from the three-compartment fit to the serum digoxin level–time data only. Initial estimates for $R_{\max}$ and K were obtained by plotting $1/R$ vs. $1/A_2$. For this fit, each data point was weighted by the reciprocal of its variance divided by the sum of the reciprocal variances for its data set (i.e., either the serum digoxin concentration data set or the ΔQS_2I data set). In this manner

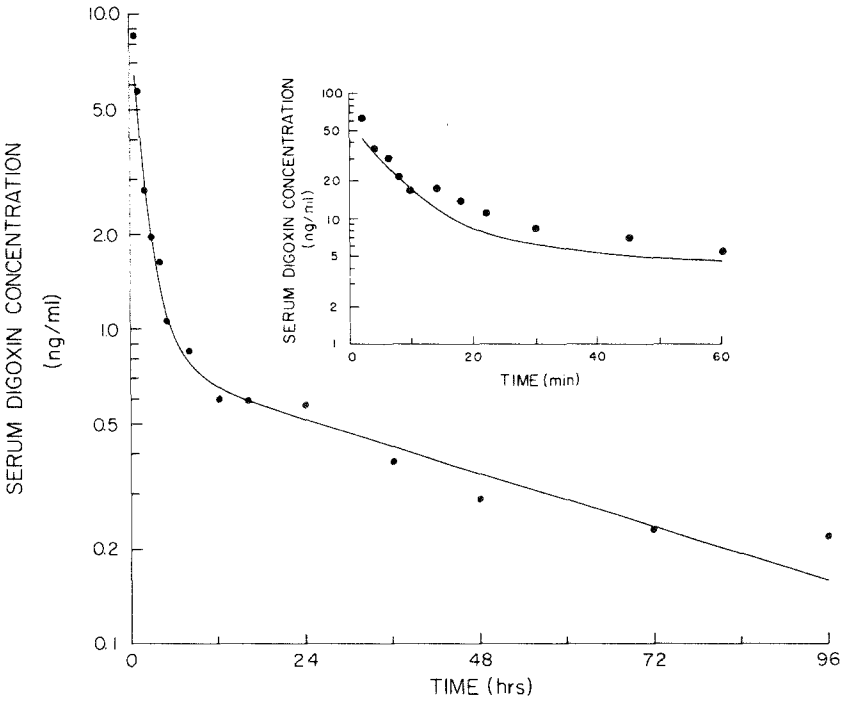


Fig. 4. Same as Fig. 2, but the curve is that predicted by the simultaneous fitting of equations 1 and 5 to both serum level and response data.

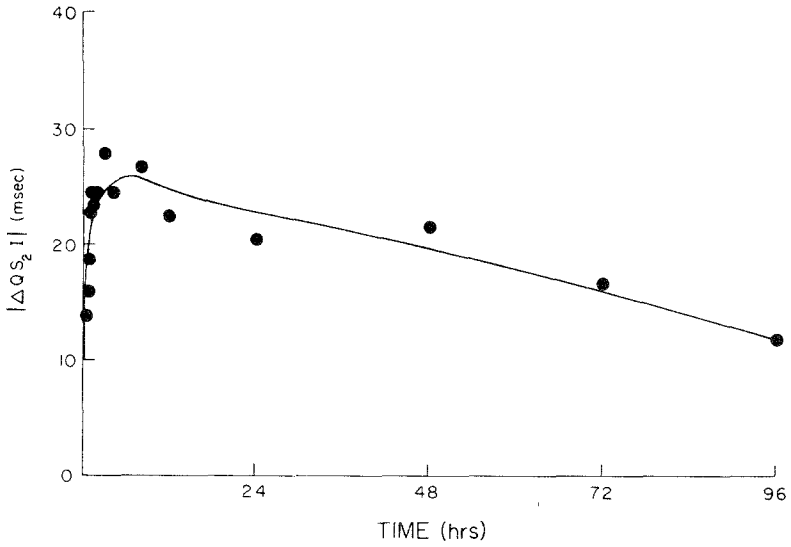


Fig. 5. Plot of averaged ΔQS_2I-time data (●) and the curve (——) for $|\Delta QS_2I|$ (response) predicted by the nonlinear regression analysis of both serum digoxin level and response data.

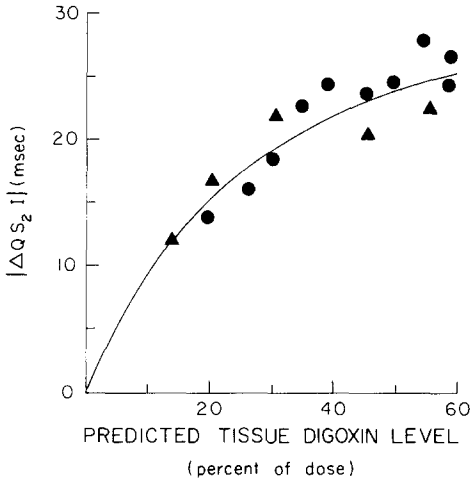


Fig. 6. Same as Fig. 3, but the tissue digoxin levels are predicted by the simultaneous fitting of equations 1 and 5 to both serum level and response data. The curve represents the relationship described by equation 5 and the constants R_{max} and K in Table II.

Table II. Nonlinear Least-Squares Estimates of the Model Parameters[a]

Parameter	Estimated by fitting	
	Serum level data only	Serum level and response data
k_{12}, hr^{-1}	1.86 (0.312)	2.12 (1.43)
k_{21}, hr^{-1}	0.060 (0.007)	0.058 (0.028)
k_{13}, hr^{-1}	4.74 (1.30)	5.75 (0.707)
k_{31}, hr^{-1}	2.30 (0.372)	1.80 (0.586)
k_{10}, hr^{-1}	0.936 (0.198)	0.910 (0.375)
V_1, liters	17.0 (3.29)	17.0 (—)
$V_{d\gamma}$, liters[b]	828. (—)	935. (—)
$t_{1/2\gamma}$, hr	36.1 (—)	41.8 (—)
Cl_T, ml/min[c]	265. (—)	258. (—)
R_{max}, msec	—	37.8 (8.24)
K, $(\%\text{-dose})^{-1}$	—	0.035 (0.033)

[a] Average body weight of the 12 subjects was 72.4 kg (7.9) and average body surface was 1.93 m^2(0.11). Standard deviations are in parentheses.
[b] Apparent volume of distribution.
[c] Total body clearance.

the sum of the weights for each data set was equal to unity and each set was able to contribute equally to the fit.

Figure 4 is analogous to Fig. 2 with the curve predicted by the simultaneous fit of both the serum level and ΔQS_2I data sets. Figure 5 contains the averaged ΔQS_2I–time data along with the curve for response obtained from the simultaneous fit. In Fig. 6 the averaged response data are plotted as a function of the corresponding digoxin levels in the deep compartment predicted by the simultaneous fit. Table II contains the final least-squares estimates of the model parameters from both fits. For the simultaneous fit the average total body clearance of digoxin was 3.6 ml/min/kg and the terminal $t_{1/2}$ value was 42 hr. This $t_{1/2}$ value is in close agreement with the average of 45 hr (four subjects) reported previously from these laboratories and obtained from a three-compartment analysis of serum digoxin level–time data (4).

DISCUSSION

In attempting to establish a relationship between drug levels and response, it is important that the relationship not be "forced" by the simultaneous fitting of both drug level–time and response–time data. When only the serum digoxin level–time data obtained in this study were fitted to the three-compartment model, an excellent fit (correlation coefficient 0.975) was obtained (Fig. 2). The levels of digoxin in the deep compartment that were predicted from this fit were very closely related to the intensity of response, as measured by the ΔQS_2I (Fig. 3). Thus in this study it was not necessary to carry out a simultaneous fit in order to clearly establish a close relationship between ΔQS_2I and digoxin levels in the deep compartment.

When both serum digoxin level–time data and ΔQS_2I–time data were fitted simultaneously, several things occurred. First the simultaneous fitting yielded a somewhat better fit to the serum digoxin–time data from 36 to 96 hr and a somewhat poorer fit to the data from 14 to 60 min (compare Figs. 2 and 4). This small degree of alteration of the fit to the serum digoxin data is to be expected since both the serum level and response data sets were weighted equally in the fitting procedure. Second, the experimental ΔQS_2I–time data were in close agreement with the theoretical curve predicted by the simultaneous analysis of response and serum level data (Fig. 5). A good fit is also indicated by the random deviations of the experimental data points about the fitted curve in Fig. 5. Third, the nonlinear relationship between ΔQS_2I and the level of digoxin in the more slowly equilibrating tissue compartment (Fig. 6) yielded a better simultaneous fit (correlation coefficient 0.970) than did any of the linear relationships that were attempted in the simultaneous fit. Although the data in Fig. 3 suggest that either a

linear or a nonlinear relationship can explain the data obtained in this study, we are in agreement with Wagner (9) that equation 5 is more reasonable because it predicts both a zero y intercept and a maximum response.

Drug level–response relationships have been reported before for the digitalis glycosides. Reuning *et al.* (10), using averaged literature data at a minimum number of points in time after i.v. dosing, demonstrated significant correlations between changes in the left ventricular ejection time index (LVET index) and predicted digoxin levels in the peripheral compartment of a two-compartment open model. A similar serum level–response relationship was also demonstrated for the QS_2 index. Weissler *et al.* (2) have shown a relationship between the i.v. dose of deslanoside C (Cedilanid) and changes in QS_2. Hoeschen and Cuddy (11) demonstrated an inverse relationship between changes in LVET and changes in serum digoxin concentration following alteration of digoxin dosage in patients on maintenance digoxin therapy. However, to date, a study designed specifically to examine the relationship between the response to digoxin and its pharmacokinetic profile, using pharmacokinetic analysis of serum concentrations and systolic time intervals measured simultaneously at intervals throughout several days after an i.v. dose, has not been reported. The results of the present study extend understanding of the relationship between serum digoxin kinetics and ΔQS_2I kinetics to the point where a close relationship between digoxin levels in the slowly distributing (deep) peripheral compartment and the inotropic response (as estimated by ΔQS_2I) is clearly established.

Although the data in this report establish a relationship between digoxin levels in the deep peripheral compartment and ΔQS_2I, several aspects related to the work require further substantiation or improvement. The variability inherent in the response measurement, both intrasubject (Fig. 1) and intersubject (standard deviations for ΔQS_2I, Table I), limits the quantitative treatment of ΔQS_2I data. Although the relationship is established for averaged data from 12 subjects, the same relationship has not been established for individual subjects. Also, the limited range of the changes in ΔQS_2I coupled with the intersubject variability makes it difficult, even for averaged data, to distinguish among different possible mathematical relationships between drug level and response. Thus the results of this study suggest that further efforts designed to develop more reproducible response measurements for digoxin would be desirable. A second aspect of the response measurement that requires further substantiation is the relationship between the ΔQS_2I and the degree of inotropy obtained after administering digoxin. Although a linear relationship has been clearly demonstrated in a previous study (1) between the ΔQS_2I and the direct measurement of the rate of pressure change in the left ventricle obtained

after digoxin administration, further studies designed to test this apparent link between ΔQS_2I and inotropy are needed. Finally, there is the possibility that metabolites of digoxin may interfere with the radioimmunoassay of digoxin serum levels if these metabolites are present in serum at a sufficient concentration. It has been demonstrated that the active metabolites digoxigenin-bisdigitoxoside and digoxigenin monodigitoxoside (12) and the inactive metabolite dihydrodigoxin (13,14) are all capable of interfering with this assay to varying degrees. It has not been established whether there are significant concentrations of these metabolites in serum because of the extremely low concentrations of assayable compounds achieved after digoxin administration in man. At present, the limits of assay technology prevent an assessment of the importance this potential contribution of metabolites to assayed digoxin levels. Further development of more specific and sensitive assays for digoxin and its metabolites would provide the opportunity to assess the degree of importance of digoxin metabolites in influencing serum level and response measurements.

If one accepts the linear relationship between inotropy and ΔQS_2I (1) and if one accepts the close relationship between digoxin levels in the deep compartment and ΔQS_2I, then any explanation of the pharmacological mechanism of digoxin-induced inotropy must accommodate the type of time profile for inotropy that is illustrated in Fig. 5. Repke *et al.* (15) have pointed out that any proposed mechanism for digitalis action must explain both the lag phase of myocardial response and the absence of a direct correlation between glycoside level in blood and glycoside effectivity. There appear to be at least two possible mechanistic explanations for lack of direct correlation of digoxin serum levels with response, the lag phase of myocardial response, and the observation in this study of an apparent correlation of inotropy with digoxin levels in the deep compartment of a three-compartment pharmacokinetic model. One possibility is that the receptor for digoxin is sufficiently remote from the digoxin in serum that the time needed for distribution to the receptor is similar to that needed for distribution to the deep body compartment. A second possibility is that the mechanism of the inotropic response to digoxin involves a sufficient delay such that the time course of inotropy is similar (perhaps fortuitously) to the time course of drug levels in the deep compartment. Certainly this second possibility should preclude the simplistic conclusion that the results of this study indicate that the receptor for digoxin is "located" in the deep peripheral compartment. Repke *et al.* (15) have presented evidence that the mechanism consisting of an inhibition of transport ATPase by cardiac glycosides followed by an increase in intracellular ionized calcium is at least consistent with the idea of a response mechanism involving considerable delay in the development of inotropy. Further research comparing the time course of digoxin levels in

serum, various tissues, and various subcellular components of the heart with the time course of inotropy is needed in order to clarify the significance of the observation in this study that inotropy, as estimated by the ΔQS_2I, is correlated with digoxin levels in the deep compartment of a three-compartment pharmacokinetic model.

APPENDIX

The equations describing the time course of drug levels in compartments 1, 2, and 3 (equations 1–3 in the text) may be rewritten in a more explicit form for the purpose of computer fitting as equations 1A, 2A, and 3A, with all symbols as defined in the text.

$$C_1 = \frac{\text{dose}}{V_1}\left[\frac{(k_{21}-\alpha)(k_{31}-\alpha)}{(\beta-\alpha)(\gamma-\alpha)}e^{-\alpha t} + \frac{(k_{21}-\beta)(k_{31}-\beta)}{(\alpha-\beta)(\gamma-\beta)}e^{-\beta t} + \frac{(k_{21}-\gamma)(k_{31}-\gamma)}{(\alpha-\gamma)(\beta-\gamma)}e^{-\gamma t}\right] \tag{1A}$$

$$A_2/\text{dose} = \frac{k_{12}(k_{31}-\alpha)}{(\beta-\alpha)(\gamma-\alpha)}e^{-\alpha t} + \frac{k_{12}(k_{31}-\beta)}{(\alpha-\beta)(\gamma-\beta)}e^{-\beta t} + \frac{k_{12}(k_{31}-\gamma)}{(\alpha-\gamma)(\beta-\gamma)}e^{-\gamma t} \tag{2A}$$

$$A_3/\text{dose} = \frac{k_{13}(k_{21}-\alpha)}{(\beta-\alpha)(\gamma-\alpha)}e^{-\beta t} + \frac{k_{13}(k_{21}-\beta)}{(\alpha-\beta)(\gamma-\beta)}e^{-\beta t} + \frac{k_{13}(k_{21}-\gamma)}{(\alpha-\gamma)(\beta-\gamma)}e^{-\gamma t} \tag{3A}$$

The symbols A, B, C, A', B', C', A'', B'', and C'' used in the text are abbreviations for the more complex preexponential factors shown in equations 1A, 2A, and 3A. The three macroconstants α, β, and γ may be defined in terms of the five microconstants (k_{ij}'s) of the model as the solution to a cubic equation, as presented by Kramer *et al.* (4), so that, with the appropriate substitutions, equations 1A, 2A, and 3A may be written solely in terms of the microconstants, dose, and V_1. Consequently, in the nonlinear least-squares fitting of equations 1 and 5 to the data, the five microconstants, R_{max}, and K were iterated, with the macroconstants calculated accordingly.

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