Inotropic Effect of Digoxin in Humans: Mechanistic Pharmacokinetic/Pharmacodynamic Model Based on Slow Receptor Binding

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Purpose. The purpose of this study was to construct a mechanistic pharmacokinetic/pharmacodynamic (PK/PD) model for digoxin that describes the relationship between plasma concentration and inotropic response.

Methods. On the basis of results obtained in the isolated perfused rat heart, a PK/PD model for digoxin in humans was developed. In fitting the model to previously published bolus dose and concentration clamp data (shortening of electromechanical systole), the plasma concentration–time curves were used as forcing functions in the computer program ADAPT II.

Results. The mechanistic approach allowed a modeling of digoxin pharmacodynamics which is consistent with available inotropic response data. The estimates of the receptor binding parameters were in the same order of magnitude as those measured *in vitro* for ouabain. The mechanistic model explained the parameters of the empirical link model (EC_{50}, E_{max} and delay time τ) in terms of the underlying processes, suggesting that the long equilibration half-time of 13 h is due to slow receptor binding. The empirical link model, in contrast, is not compatible with a noninstantaneous receptor binding process and led to estimates of the delay time τ that were dependent on the digoxin administration schedule.

Conclusions. The new, mechanistic model may provide a rationale for better understanding of digoxin pharmacodynamics and could become a tool to bridge the gap between *in vitro* and *in vivo* studies.

KEY WORDS: digoxin; humans; model; pharmacodynamics; pharmacokinetics.

INTRODUCTION

Although cardiac glycosides have been used for more than 200 years, they are still a mainstay in the treatment of congestive heart failure (1,2). Thus, digoxin remains one of the most commonly prescribed of all cardiac medications. It is now well-accepted that the positive inotropic effects of digoxin and related cardiac glycosides on cardiac muscle are mediated through inhibition of Na^+/K^+-ATPase (sodium pump) by binding to a specific extracytoplasmic site of the α-subunit of this enzyme. Via the sodium gradient-coupled Na^+/Ca^{2+} exchanger, this increases intracellular Ca^{2+} availability for contractile proteins. This positive inotropic effect is the basis of the therapeutic use of cardiac glycosides in the management of congestive heart failure.

In pioneering work, Gold *et al.* (3) first studied the effect kinetics of digoxin in humans. Pharmacokinetic/pharmacodynamic (PK/PD) models were developed much later by Kramer et al. (4) and Kelman and Whiting (5) who linked the time course of positive inotropic effect to digoxin amount in peripheral or effect compartment(s), respectively. These empirical link models do not explain the role of cardiac uptake and receptor binding kinetics in determining the long equilibration delay between drug concentration and inotropic effect.

However, despite significant need for a better understanding the pharmacodynamics of digoxin in humans in view of the very narrow therapeutic range, a mechanistic PK/PD model for digoxin in humans is still lacking.

Based on experiments in the isolated perfused rat heart, we have recently developed a mechanistic PK/PD approach for digoxin that explicitly models transport to the receptors (sodium pumps) and the dynamics of drug–receptor interaction linking the observed inotropic effect to receptor occupation (6). However, the limited information that can be obtained from *in vivo* experiments in humans would not allow the identification of such a more detailed model. One way to overcome this problem is complexity reduction; that is, the investigation of relevant subsystems and incorporation of the results in the model applied *in vivo* (7). As the organs represent the natural subsystems of the body, experiments in isolated perfused organs offer an efficient way to develop more detailed PK/PD models that provide insight into underlying processes and can ultimately be scaled-up from the animal organ to the whole body level in humans. In applying this mechanistic approach to data obtained in healthy volunteers (4,8), the results offer an explanation for 1. the long temporal delay between plasma concentrations and effect, which characterizes the pharmacodynamics of digoxin in the transient state, and 2. the inconsistencies in parameter estimates obtained with the link model for different experimental designs.

METHODS

Data

We analyzed data from two PK/PD studies with different experimental designs. One is the classical PK/PD study of digoxin where plasma concentration–time data and the shortening of the electromechanical systole corrected for heart rate (ΔQS_2c) were measured after administration of a 1.0 mg i.v. bolus injection in 12 healthy male volunteers (4); 23 serum digoxin samples and 15 ΔQS_2c measurements were obtained over a 4-day period. Furthermore, we analyzed the ΔQS_2c-response observed in a "concentration-clamp" (i.e., step-response) experiment where an approximate plateau value of ~4.2 ng/ml digoxin was established over 4 h (8). In order to generate this plateau concentration in less than 15 min, digoxin was administered by controlled infusion in six healthy male volunteers: a bolus injection of 100 μg (to fill the central compartment) was followed by a constant rate plus a biexponentially decreasing rate infusion (to compensate elimination and distribution, respectively). The underlying PK parameters of digoxin were taken from the PK study of Kramer

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et al. (4). The concentration–time (8 samples) and step response–time curve (16 measurements) was observed over a 4-h period. The response data sets (average values) obtained with these differently designed experiments were fitted simultaneously to improve parameter estimation as both experiments provide information on the system response at different time scales (4 h and 4 days, respectively).

Mechanistic Receptor-Binding Model

Whereas in the rat heart consecutive inhibition of first α_2- and then α_1-isoform of the Na^+/K^+-ATPase (high affinity/low capacity and a low affinity/high capacity binding sites, respectively) mediates the positive inotropic effect of digoxin with increasing dose (6,9), the isoforms expressed in the human heart have a similar affinity, and binding kinetics can be approximated by a single-site model (10,11). Accordingly, our model developed for the rat heart (6) could be simplified to a one-receptor model shown in Fig. 1, where Q_D is the functional coronary flow and $C(t)$ the plasma digoxin concentration; the subscripts *vas* and *is* denote vascular and interstitial compartment, respectively. The corresponding differential equations are

$$dD_{vas}(t)/dt = -(Q_D/V_{vas} + k_{vi})\, D_{vas}(t) + k_{iv}\, D_{is}(t) + Q_D\, C(t) \quad (1)$$

$$dD_{is}(t)/dt = k_{vi}\, D_{vas}(t) - \{k_{iv} + k_{on}\,[R_{tot} - DR(t)]\}D_{is}(t) + K_{off}\, DR(t) \quad (2)$$

$$dDR(t)/dt = k_{on}\,[R_{tot} - DR(t)]\, D_{is}(t) - k_{off} DR(t) \quad (3)$$

The volume of the vascular compartment, $V_{vas} = 0.06$ ml/g (12), coronary blood flow, 70 ml/min per 100 g (13), and heart weight, 300 g, are taken from anatomic data. The functional flow for digoxin transport into the heart is calculated from plasma flow (45 ml/min per 100g) and the unbound fraction of digoxin in plasma (75%) as Q_D ~100 ml/min. The rate constants k_{vi} and k_{iv} describing passive transport across the capillary wall are fixed to the values estimated in the rat heart ($k_{vi} = 41.2\ min^{-1}$, $k_{iv} = 8.91\ min^{-1}$) (6) assuming that the increase in the apparent permeability surface-area or permeation clearance, $CL_{vi} = k_{vi}V_{vas}$, is mainly due to the higher vascular volume. The binding probability of digoxin in the interstitial space, D_{is}, to the saturable binding site R is dependent on the association rate constants k_{on} (in units of $1{\cdot}min^{-1}{\cdot}nmol^{-1}$) and the amount of free membrane receptors, which is equal to $R = [R_{tot} - DR(t)]$; where R_{tot} is the unknown amount of available receptor sites, and DR denotes the receptor–digoxin complexes (i.e., amount of bound digoxin). This leads to a time-dependent factor $K_{on}(t) = k_{on}\,[R_{tot} - DR(t)]$. The rate constant for the dissociation of the bound ligand was denoted by k_{off} (in units of 1/min); $K_D = k_{off}/k_{on}$ and $K_A = 1/K_D$ represent the equilibrium dissociation and affinity constants, respectively. Note that the above distribution model also accounts for instantaneous nonspecific binding of digoxin.

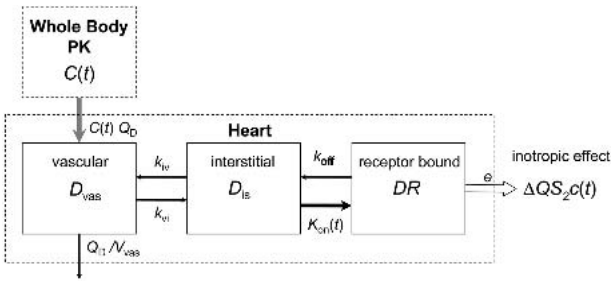

Fig. 1. Mechanistic model of digoxin pharmacodynamics. The heart model includes a vascular, interstitial, and receptor-binding compartment. First-order rate constants of transcapillary transport are denoted by k_{vi} and k_{iv}. $K_{on}(t)$ is the fractional rate for saturable receptor binding, while k_{on} and k_{off} are the association and dissociation constants, respectively. The curve describing digoxin plasma concentration, $C(t)$, provides the digoxin input rate $Q_DC(t)$, where Q_D denotes the functional coronary blood flow. The effect is proportional to receptor occupation with scaling factor e.

The effect, i.e., inotropic response $E(t)$, was linked to the number of receptors occupied by drug (DR),

$$E(t) = e\, DR(t) \quad (4)$$

where the parameter e is the effect per unit of digoxin–receptor complex analogous to "efficacy" parameter in receptor theory (14). Thus, the model contains four free parameters; namely, k_{on}, k_{off}, R_{tot}, and e. The effect $E(t)$ is the observed shortening of the electromechanical systole, ΔQS_2c; that is, $E(t) = \Delta QS_2c - \Delta QS_2c_0$ where QS_2c_0 denotes the placebo effect (baseline response).

At steady state, the changes in compartmental digoxin amounts vanish [$dD_{vas}(t)/dt = dD_{is}\ (t)/dt = dDR(t)/dt = 0$ in Eqs. (1)–(3)], and the solution of the equations together with Eq. (1) can be used to predict the concentration–response curve,

$$E_{ss} = \frac{e\, R_{tot} C_{ss}}{K_D(k_{iv}/CL_{vi}) + C_{ss}} \quad (5)$$

Thus, a hyperbolic E_{max} model [$E_{ss} = E_{max}C_{ss}/(EC_{50} + C_{ss})$] is obtained with parameters

$$E_{max} = e\, R_{tot} \quad (6)$$

$$EC_{50} = \frac{K_D k_{iv}}{k_{vi} V_{vas}} \quad (7)$$

Note that the EC_{50} increases proportionally as K_D or the equilibrium partitioning ratio k_{vi}/k_{iv} increase.

Notably, for the concentration-clamp experiment, where following the concentration step at $t = 0$ the plateau $C(t) = C(0) = C_0$ is maintained during the time course of the experiment, the effect time course can be described by an explicit function when transcapillary exchange is not rate-limiting [solution of Eqs. (2) and (3) and substitution of Eq. (4)],

$$E_{step}(t) = E_{ss}[1 - \exp(-t/\tau_{step})] \quad (8)$$

where E_{ss} is given by Eq. (5) and

$$\tau_{step} = \frac{1}{k_{on} D_{is,0} + k_{off}} \quad (9)$$

Note that $D_{is,0}$ is obtained from from Eq. (1) for $dD_{vas}(t)/dt = 0$,

$$D_{is,0} = (k_{vi}/k_{iv}) V_{vas} C_0 \qquad \text{for } t \geq 0 \quad (10)$$

This approximation [Eq. (8)] is valid for $\tau_{step} \gg 1/k_{vi}$. Interestingly, the time constant characterizing the step response is dose-dependent (i.e., dependent on the concentration plateau). Furthermore, τ_{step} approaches $1/k_{off}$ for decreasing serum concentrations ($D_{is,0} \to 0$).

Empirical Link Model

The results obtained with the mechanistic model will be compared with the predictions of the empirical link model. In this traditional PK/PD model (15,16), a hypothetical biophase concentration $C_B(t)$ is generated from the plasma concentration $C(t)$ using a first-order delay with time constant τ, $C_B(t) = C(t)*e^{-t/\tau}$, where * denotes convolution. This operation can be simply accomplished by solving the differential equation

$$\frac{dC_B}{dt} = \frac{1}{\tau}[C(t) - C_B(t)] \tag{11}$$

Assuming instantaneous receptor binding, the time course of inotropic effect $E(t)$ is then linked to the biophase concentration $C_B(t)$ using the hyperbolic E_{max} model:

$$E(t) = \frac{E_{max}C_B(t)}{EC_{B,50} + C_B(t)} \tag{12}$$

E_{max} is the maximum induced response, and EC_{50} is the concentration producing 50% of E_{max}.

It follows from Eq. (11) that in a concentration-clamp experiment, the resulting step response of biophase concentration is given by $C_B(t) = C_0 (1 - e^{-t/\tau})$, and Eq. (12) can be written explicitly

$$E_{step}(t) = \frac{E_{max}C_0(1 - e^{-t/\tau})}{EC_{B,50} + C_0(1 - e^{-t/\tau})} \tag{13}$$

It is obvious that the step response predicted by the empirical model [Eq. (13)] is not compatible with that of the mechanistic model [Eq. (8)]. Only under the assumption of a linear E-C_B model (i.e., for $C_0 \ll EC_{50}$) we obtain an exponential function:

$$E_{step}(t) \approx (E_{max}/EC_{B,50})C_0(1 - e^{-t/\tau}) \tag{14}$$

Data Analysis

For the receptor binding model, Eqs. (1)–(3) were solved and Eq. (4) was fitted to the pharmacodynamic data. Thereby, the respective $C(t)$-curves were incorporated as forcing functions: The bolus dose disposition $C(t)$-data of Kramer *et al.* (4) were refitted by a tri-exponential function leading to $C(t) = 57.3e^{-0.164t} + 9.99e^{-0.011t} + 0.74e^{-0.00024t}$ (C in ng/ml, t in units of min) whereas the concentration-clamp $C(t)$-data of Weiss et al. (8) were used directly [plateau value of $C(t)$ ~4.2 ng/ml for $0 < t < 4$ h). For each model, the mean ΔQS_2c data of both experiments were fitted simultaneously using the ADAPT II-software package (17). Maximum likelihood estimation was performed assuming that the measurement error has a standard deviation which is a linear function of the measured quantity. Such simultaneous analysis of two or more experimental data sets obtained for different dosing schedules improves the ability to resolve unique estimates of parameters. The Akaike information criterion (AIC) was used to compare the acceptability of models (17). The step response data (8) were also separately fitted by Eq. (8) in order to test the validity of the mechanistic model and to estimate the parameter τ_{step}. Finally, both experimental data sets and ΔQS_2c data simulated with the mechanistic model were fitted using the link model [Eq. (12) after solving the differential Eq. (11) numerically] in order to explain the role of the rate constants of the mechanistic model in determining concentration–effect hysteresis (delay time constant τ) in the link model.

RESULTS

Figures 2A and 2B show the average $\Delta QS_2c(t)$-data of the bolus injection and concentration-clamp experiment, respectively, together with the lines obtained by a simultaneous fit obtained with the mechanistic model. The model provided a good fit to both data sets, with parameter estimates $k_{on} = 0.26\ 10^{-4}$ min^{-1}·ng^{-1}, $k_{off} = 0.56\ 10^{-3}$ min^{-1}, $R_{tot} = 134.7$ ng, and $e = 0.23$ ms/ng. However, the low parameter sensitivity for R_{tot} and the extremely large values of the approximate coefficients of variation indicated that a reliable estimation of R_{tot} was not possible with these data. Thus, based on the Na^+/K^+-ATPase concentration of ~700 pmol/g wet weight measured in normal human left ventricular myocardium (18), R_{tot} was fixed to the corresponding value of 162 ng. Very similar parameter estimates $k_{on} = 0.22\ 10^{-4}$ min^{-1}·ng^{-1}, $k_{off} = 0.49\ 10^{-3}$ min^{-1}, and $e = 0.19$ ms/ng were then obtained with approximate coefficients of variation of 12.1%, 12.3%, and 5.4%, respectively. Thus, an equilibrium dissociation constant of $K_D = 22.3$ ng is obtained for digoxin receptor bind-

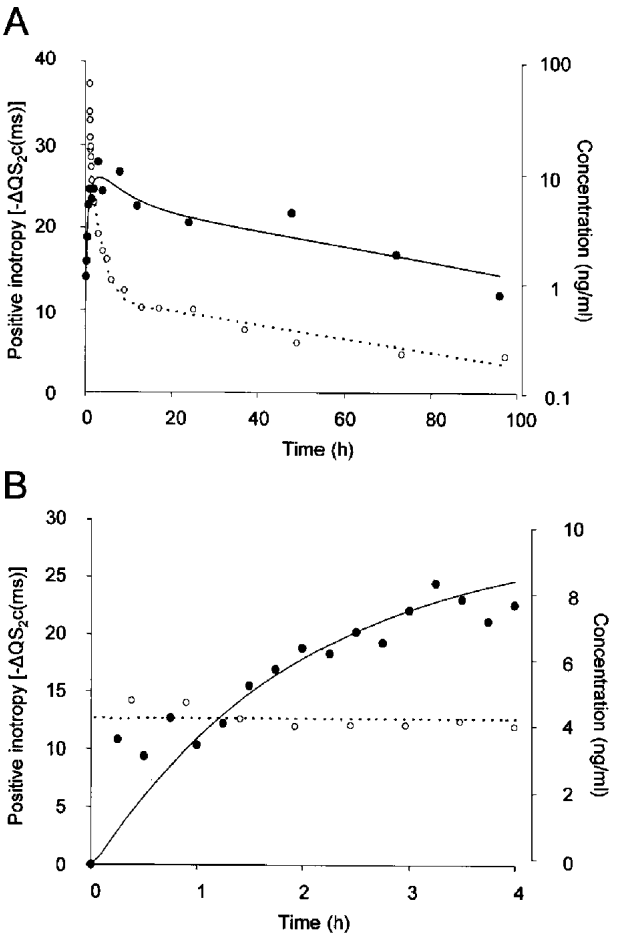

Fig. 2. Simultaneous fit of the mechanistic model to average inotropic response data of digoxin obtained after bolus dose (A) and concentration-clamp (B) experiments. The dotted lines indicate the underlying concentration–time curves. The plasma concentration (○) and response (●) data in panels A and B were taken from work of Kramer et al. (4) and Weiss et al. (8), respectively.

ing. This implies that the steady-state concentration–response [Eq. (5)] curve is characterized by parameters EC_{50} = 0.27 ng/ml and E_{max} = 30.9 ms. As expected, the step response function [Eq. (8)] described the average $\Delta QS_2c(t)$-data of the concentration-clamp experiment reasonably well (Fig. 3). The time constant τ_{step} = 1.3 h estimated directly by fitting Eq. (8) to the step response data is in the same order of magnitude as the value of 2.1 h predicted by Eq. (9) after substituting the above estimates of k_{on} and k_{off} as well as $D_{is,0}$ [Eq. (10)].

As expected from the previous applications (4,5,15), the empirical link model fitted the bolus dose data equally well (fit not shown). For the EC_{50} (i.e., the concentration required to achieve 50% of the maximum decrease in the electromechanical systole, ΔQS_2c), a value of EC_{50} = 0.41 ng/ml was estimated. The corresponding maximum value was E_{max} = 34.8 ms. The time constant underlying the first-order delay of τ = 18.7 h corresponds to an equilibration half-life of 13 h. The parameter estimates were substituted into Eqs. (5) and (12) to predict the steady-state concentration–effect relationships for both models (Fig. 4).

In contrast to the reasonable parameter estimates obtained in fitting the bolus dose data, however, its application to the step response data (fit not shown) led to unrealistic high E_{max} and EC_{50} estimates [indicating a collapse of Eq. (13) to a linear relationship Eq. (14)] and 10-fold lower τ value of 1.5 h (similar to the estimate, τ_{step} = 1.3 h, mentioned above).

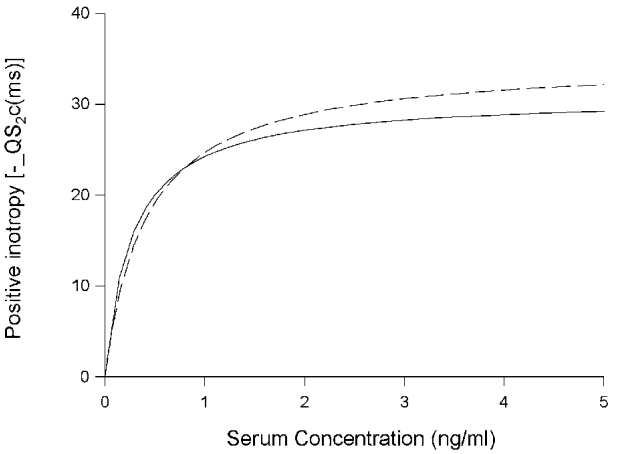

Fig. 4. Steady-state concentration–effect curves of digoxin in healthy volunteers predicted with the mechanistic (——) and empirical link models (– – –) with EC_{50} values of 0.27 and 0.41 ng/ml, respectively.

DISCUSSION

A novel mechanistic approach based on drug-receptor binding kinetics is used to analyze the inotropic response data of digoxin following two different administration schedules. The results are compared with those obtained with the empirical link model. The data came from a bolus dose (4) and a concentration-clamp (8) experiment, respectively, in healthy male volunteers. Our mechanism-based PK/PD model offers for the first time a quantitative explanation of the long temporal delay between plasma concentrations of digoxin and the inotropic effect following a bolus dose as estimated with the link model (equilibration half-time of 13 h). Modeling suggests that the observed hysteresis is due to slow receptor binding and not caused by transport to the site of action (i.e., the contributions of convective transport and transcapillary exchange are negligible in this case). This is illustrated in Fig. 5, where the change in the delay time τ with $1/k_{on}$ or $1/k_{vi}$, respectively, is depicted. (τ was estimated with the link model using data simulated for various k_{on} or k_{vi} values with the mechanistic model, keeping K_D and k_{vi}/k_{iv} constant.) This is in accordance with the observation that the acute myocardial uptake of digoxin in humans is much faster than the onset of the inotropic effect (19). Although it is generally accepted that receptor association and dissociation processes of cardiac glycosides are slow, *in vitro* results from human hearts are only available for ouabain where in the presence of K^+, k_{off} and k_{on} values of about 4 10^{-3} min^{-1} and 0.3 10^{-4} min^{-1} nM^{-1} (K_D = 125 nM) have been observed (20); our estimates are in the same order of magnitude. [Note that in digitalis-insensitve species, like the rat, binding processes are much faster (6).] Based on our parameter estimates in healthy volunteers, the therapeutic concentration range of 0.5 to 1.5 ng/ml digoxin (1) produces an effect between 65 and 85% of the maximum inotropic effect [Eq. (5)]. Note that the

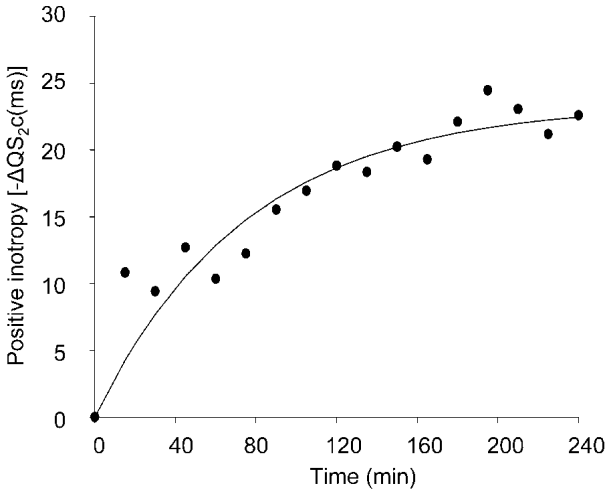

Fig. 3. Fit of the function $DE_{ss}[1 - \exp(-t/\tau_{step})]$ [Eq. (11)] to time course of inotropic response obtained after the concentration-clamp experiment (8).

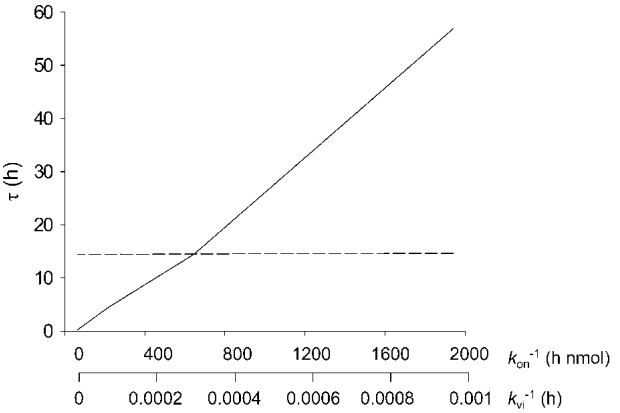

Fig. 5. Delay time constant τ estimated with the link model from data simulated using the mechanistic model when k_{on} (——) or k_{vi} (– – –) was varied, respectively, from one-third to 10-fold of the normal value, keeping K_D (or k_{vi}/k_{iv}) constant.

meaning of parameter R_{tot} remains unclear in the current context because the term "receptor" as used here is mainly based on the ability of the model to predict the time course of the inotropic effect. Furthermore, because digoxin acts via inhibition of Na^+/K^+-ATPase, a downregulation of sodium pump expression in the failing heart increases the sensitivity to inotropic stimulation (2). This has to be considered in discussing the role of the parameter R_{max} defined in our model. It is important to note that we have also tested a model with linear receptor-binding which, however, gave a worse fit ($\Delta AIC = 45$).

It should be emphasized that due to the use of average data and the mismatch between model complexity and the limited information content of the data, the current approach resembles the methodology of "forward modeling" (21,22); that is, the construction of a model that provides quantitative insight into the underlying mechanisms rather than an exact evaluation of all parameters was the principal goal of our approach. Initially, four preassigned parameters had to be incorporated using physiological/anatomical parameters (Q_D, V_{vas}) and estimates obtained in the rat heart (k_{vi}, k_{iv}) to ensure that the number of adjustable parameters, k_{on}, k_{off}, R_{tot}, and e, could be kept to a minimum. The additional assumption of a R_{tot}-value led to small changes in the parameters k_{on}, k_{off}, and e and provided the approximate coefficients of variation of these estimates. Regardless of the limitation, the current approach should be still meaningful because we could demonstrate that the mechanistic model, in contrast to the link model, is in accordance with available data: it captured the response observed in humans for two different administration schedules, and the steady-state predictions of the model were in agreement with the therapeutic concentration range of digoxin. Especially the model's capability to predict the time course of step response (i.e., the observed time constant τ_{step}) in terms of the intrinsic model parameters k_{on} and k_{off} [Eq. (9)] is encouraging. Alternative models, including those with a time-dependent effectuation process (see Ref. 23 for a recent review), have been tested but did not improve the fit. Note that the observed nonlinearity [i.e., dose dependency of τ_{step}, Eq. (9)] excludes the possibility that time lags in post-receptor events could account for the delayed effect development.

The results obtained with the empirical link model shed some new light on the limitations of this conventional PK/PD modeling approach. Due to the dependence of parameter estimates from the experimental design (administration schedule), no unique set of model parameters could be estimated. The empirical model described the bolus dose data equally well, with an equilibration half-time of 13 h, which is in agreement with the value of 14 h estimated by Holford and Sheiner (15) in reanalyzing the data of Kramer *et al.* (4), but differs from the value of 4 h obtained by Kelman and Whiting (5) based on experiments with a shorter observation period (sampling up to 12 h). However, the parameter estimates obtained by fitting the step-response data were unrealistic. This can be explained by the inconsistency between the observed exponential function (Fig. 3) and prediction of the link model [Eq. (13)]. Note that the assumption of a linear E-C_B relationship [Eq. (14)] does not solve this problem because, as shown above, the resulting estimate $\tau_{step} = 1.3$ h is then 10-fold lower than that of 18.7 h obtained in fitting the bolus dose data. Thus, the estimate of $\tau = 18.7$ h only empirically describes the hysteresis observed for a specific digoxin disposition curve. This dose dependency of the apparent equilibration time, τ, is not surprising in view of the fact that a linear transformation [Eq. (11)] has been used to describe the nonlinear transient process of saturable receptor binding. Note that an input rate dependence of PD parameter estimates has been observed for several other drugs (24). While a linear $C_B(t)$ - $E(t)$ relationship has been previously used to analyze the effect time course of digoxin with the link model (5,15), our results are in favor of the E_{max} model, not only because of the lower AIC-value, but also because of its correspondence to the receptor-binding model. Note that the delay time constant of 23 min reported by Forester *et al.* (25) simply reflects effect onset within 1 h after bolus dose of 1.6 mg digoxin. Interpreted as a τ_{step}-value, it would correspond to a step response caused by a functional average serum concentration of C_0 ~17 ng/ml [Eq. (12)].

It is of practical importance, however, that the conceptual shortcomings of the empirical link model [and of the "deep compartment" approach used in the pioneering work of Kramer *et al.* (4)] do not diminish the usefulness of these models for simulation purposes. Thus, the role of delayed response in determining the digoxin effect has already been explained for various modes of administration [e.g., schedule, rate and route (26,27)]. It is shown in Fig. 6 that the prediction provided by the empirical model for the response to multiple oral dosing is quite similar to that of the mechanistic model.

As already pointed out in the introduction, we call the link model "empirical" because both its usefulness and explanatory power are limited. The meaning of the intermediate "biophase concentration" $C_B(t)$ in Eq. (2) remains obscure in the case of slow receptor binding: it is obviously not the digoxin concentration in the vicinity of receptors (i.e., the site at which the drug exerts its action) as the slow receptor binding is the main determinant of τ. Due to the potential effect of model misspecification, the empirical model may lead to biased estimates of E_{max} and EC_{50} when applied to non-steady-state data. It appears, however, that the assumption of rapid receptor binding may be valid for most drugs; up to now there is only one other example where receptor binding is the rate-

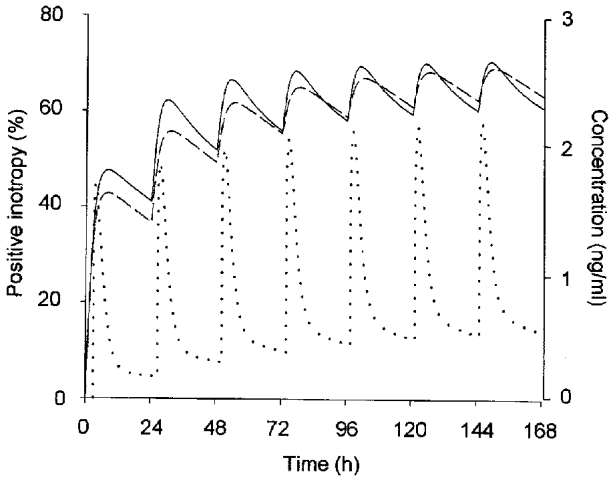

Fig. 6. Inotropic response to multiple dosing of digoxin (0.25 mg/day) simulated with the mechanistic (——) and empirical (– – –) model, respectively, assuming a three-exponential disposition function (4) and first-order absorption ($k_a = 0.6$ h^{-1}).

limiting process, namely, the binding of calcium antagonists to ion channels (28).

This analysis resolves a long-standing question. Thus, Atkinson (29) in discussing the elegant work of Gold *et al.* (3), stated, "I suspect, but have no proof, that the process of digoxin distribution from plasma to its myocardial site of action is responsible for this clinically important delay." While this may be true for most drugs, it does not hold for digoxin where slow binding accounts for the delay.

With advancements in molecular biology, more detailed information on the mechanism of digoxin action becomes available. We suggest that mechanistic PK/PD models are valuable tools to bridge the gap between studies at the molecular level and the functioning of organ systems *in vivo*.

REFERENCES

1. P. J. Hauptman and R. A. Kelly. Digitalis. *Circulation* **99**:1265–1270 (1999).
2. K. Kjeldsen, A. Norgaard, and M. Gheorghiade. Myocardial Na,K-ATPase: the molecular basis for the hemodynamic effect of digoxin therapy in congestive heart failure. *Cardiovasc. Res.* **55**: 710–713 (2002).
3. H. Gold, Mc. K. Cattell, T. Greiner, L. W. Hanlon, N. T. Kwit, W. Modell, E. Cotlove, J. Benton, and H. L. Otto. Clinical pharmacology of digoxin. *J. Pharmacol. Exp. Ther.* **109**:45–57 (1953).
4. W. G. Kramer, A. J. Kolibash, R. P. Lewis, M. S. Bathala, J. A. Visconti, and R. H. Reuning. Pharmacokinetics of digoxin: relationship between response intensity and predicted compartmental drug levels in man. *J. Pharmacokinet. Biopharm.* **7**:47–61 (1979).
5. A. W. Kelman and B. Whiting. Modeling of drug response in individual subjects. *J. Pharmacokin. Biopharm.* **8**:115–130 (1980).
6. W. Kang and M. Weiss. Digoxin uptake, receptor heterogeneity and inotropic response in the isolated rat heart: a comprehensive kinetic model. *J. Pharmacol. Exp. Ther.* **302**:577–583 (2002).
7. D. E. Mager and W. J. Jusko. Pharmacodynamic modeling of time-dependent transduction systems. *Clin. Pharmacol. Ther.* **70**: 210–216 (2001).
8. M. Weiss, W. Sziegoleit, A. Fahr, and W. Förster. Rapid achievement of a serum concentration plateau of digoxin through controlled infusion. *Eur. J. Clin. Pharmacol.* **25**:455–457 (1983).
9. A. Schwartz and N. Petrashevskaya. The importance of calcium in interpretation of NaK-ATPase isoform function in the mouse heart. *Cardiovasc. Res.* **51**:9–12 (2001).
10. A. A. McDonough, J. Wang, and R. A. Farley. Significance of sodium pump isoforms in digitalis therapy. *J. Mol. Cell. Cardiol.* **27**:1001–1009 (1995).
11. J. Wang, J. B. Velotta, A. A. McDonough, and R. A. Farley. All human Na(+)-K(+)-ATPase alpha-subunit isoforms have a similar affinity for cardiac glycosides. *Am. J. Physiol. Cell Physiol.* **281**:C1336–C1343 (2001).
12. G. P. Dobson and J. H. Cieslar. Intracellular, interstitial and plasma spaces in the rat myocardium in vivo. *J. Mol. Cell. Cardiol.* **29**:3357–3363 (1997).
13. A. C. Guyton. *Textbook of Medical Physiology,* 8th ed. W.B. Saunders Company, Harcourt Brace Jovanovich, Inc., Philadelphia, London, Toronto, Montreal, Sydney, Tokyo, 1991.
14. T. Kenakin. *Pharmacologic Analysis of Drug-Receptor Interaction.* Raven Press, New York, 1993.
15. N. H. Holford and L. B. Sheiner. Kinetics of pharmacologic response. *Pharmacol. Ther.* **16**:143–166 (1982).
16. D. D. Breimer and M. Danhof. Relevance of the application of pharmacokinetic-pharmacodynamic modeling concepts in drug development. The "wooden shoe" paradigm. *Clin. Pharmacokinet.* **32**:259–267 (1997).
17. D. Z. D'Argenio and A. Schumitzky. *ADAPT II User's Guide: Pharmacokinetic/Pharmacodynamic Systems Analysis Software.* Biomedical Simulations Resource, Los Angeles, 1997.
18. T. A. Schmidt, P. D. Allen, W. S. Colucci, J. D. Marsh, and K. Kjeldsen. No adaptation to digitalization as evaluated by digitalis receptor (Na,K-ATPase) quantification in explanted hearts from donors without heart disease and from digitalized recipients with end-stage heart failure. *Am. J. Cardiol.* **71**:110–114 (1993).
19. A. C. Powell, J. D. Horowitz, Y. Hasin, M. L. Syrjanen, S. Horomidis, and W. J. Louis. Acute myocardial uptake of digoxin in humans: correlation with hemodynamic and electrocardiographic effects. *J. Am. Coll. Cardiol.* **15**:1238–1247 (1990).
20. L. G. Lelievre, G. Crambert, and P. D. Allen. Expression of functional Na,K-ATPase isozymes in normal human cardiac biopsies. *Cell. Mol. Biol.* **47**:265–271 (2001).
21. M. M. Graham. Model simplification: complexity versus reduction. *Circulation* **72**:IV63–IV68 (1985).
22. R. Mukkamala and R. J. Cohen. A forward model-based validation of cardiovascular system identification. *Am. J. Physiol.* **281**:H2714–H2730 (2001).
23. D. E. Mager, E. Wyska, and W. J. Jusko. Diversity of mechanism-based pharmacodynamic models. *Drug Metab. Disp.* **31**:510–518 (2003).
24. G. Castaneda-Hernandez, G. Caille, and P. du Souich. Influence of drug formulation on drug concentration-effect relationships. *Clin. Pharmacokinet.* **26**:135–143 (1994).
25. W. Forester, R. P. Lewis, A. M. Weissler, and T. A. Wilke. The onset and magnitude of the contractile response to commonly used digitalis glycosides in normal subjects. *Circulation* **49**:517–521 (1974).
26. A. J. Kolibash Jr., R. P. Lewis, D. W. Bourne, W. G. Kramer, and R. H. Reuning. Extension of the serum digoxin concentration-response relationship to patient management. *J. Clin. Pharmacol.* **29**:300–306 (1989).
27. R. P. Lewis. Clinical use of serum digoxin concentrations. *Am. J. Cardiol.* **69**:97G–107G (1992).
28. S. Shimada, Y. Nakajima, K. Yamamoto, Y. Sawada, and T. Iga. Comparative pharmacodynamics of eight calcium channel blocking agents in Japanese essential hypertensive patients. *Biol. Pharm. Bull.* **19**:430–437 (1996).
29. A. J. Atkinson. A pharmacokinetic odyssey. *The Pharmacologist* **31**:229–234 (1989).