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Research Article

An Experimental Design Strategy for Quantitating Complex Pharmacokinetic Models: Enterohepatic Circulation with Time-Varying Gallbladder Emptying as an Example

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A four-step strategy is proposed for determining appropriate experimental designs for investigating the pharmacokinetics of drugs characterized by complex compartmental models and this strategy has been applied to the pharmacokinetics of enterohepatic circulation (EHC). The four steps are (1) to establish an appropriate pharmacokinetic model, (2) to complete an identifiability analysis for the model to determine the route(s) of administration and sampling compartment(s) that are theoretically adequate for the quantitation of model parameters, (3) to carry out nonlinear least-squares fitting for the proposed number and timing of simulated error-free data points, and (4) to complete nonlinear least-squares fits of the model to data obtained by adding random error to the simulated data in step 3. The four-compartment model chosen for EHC of unchanged drug contained central, peripheral, gallbladder, and intestinal compartments and an intermittent gallbladder emptying rate constant. Identifiability analysis demonstrated that three alternative experimental designs for route(s) of administration and sampling compartment(s) are adequate for quantitating all model parameters, when the gallbladder emptying rate constant as a function of time is known (using controlled emptying from an engineered gallbladder in an animal model or quantitation in humans or animals using imaging techniques). Parameter estimates from fitting error-free data matched closely with the known values for all three experimental designs, indicating an adequate number and appropriate timing of data points. Results from fitting simulated data containing ±10% random error indicated unacceptable coefficients of variation and a nonrandom pattern in residual plots for one of the experimental designs. Of the two remaining designs, one was less resilient relative to poor initial estimates and relative to timing of gallbladder emptying simultaneously with the distribution process. It is clear that application of this new strategy permits the elimination of experimental designs that are inadequate (from either a theoretical or an experimental standpoint) prior to initiating *in vivo* experiments. As such, it represents a major advance in reliability over methods used previously for complex models.

KEY WORDS: pharmacokinetics; experimental design; enterohepatic circulation; intermittent gallbladder emptying; simulation; compartmental model; nonlinear least-squares fitting.

INTRODUCTION

Complex pharmacokinetic models, composed of four or more compartments, have generally not been amenable to quantitation of the pharmacokinetic constants describing the model. The cause of this is the inadequacy of the techniques used to analyze data for a typical two- or three-compartment mammillary model when applied to more complex models. Although some authors have successfully fit time-dependent data using a complex pharmacokinetic model (1–4), the reliability of the estimates of the microconstants has not been assessed and the validity of the models has not been verified experimentally. The objective of this study is to demonstrate a new approach to the problem of obtaining reliable estimates of pharmacokinetic parameters for a complex model.

Drugs which undergo enterohepatic circulation (EHC) typify the difficulty connected with pharmacokinetic analysis when data can be obtained only for a limited number of compartments in a complex pharmacokinetic model. In addition, the intermittent nature of gallbladder emptying further complicates the pharmacokinetic analysis. Although the donor–recipient animal model (5–7) is commonly used in studies of EHC, it is more important for qualitative demonstration of the presence of EHC than it is for quantitative evaluation of the pharmacokinetic rate constants for EHC of the tested drugs. In previous work from this laboratory (8–10) and from others (10–13), complex pharmacokinetic models for EHC have been used to obtain integrated equations for bioavailability, clearance, and half-life. However, these models are yet to be applied to an analysis of time-dependent compartmental data yielding the pharmacokinetic parameters for the particular model. Guidelines for designing an experiment for the reliable evaluation of the contribution of the individual steps of a model of EHC (or individual steps in

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any complex pharmacokinetic model) to *in vivo* drug disposition are yet to be established.

A four-step strategy is proposed in this study for designing pharmacokinetic experiments for the investigation of complex models, such as an EHC model with intermittent gallbladder emptying. In the first step, a pharmacokinetic model for drugs that undergo EHC in unchanged form is proposed. This model includes an intermittent process, i.e., gallbladder emptying, and different compartments for gallbladder, intestine, and plasma. The second step is the use of identifiability analysis, a method for determining the necessary route(s) of administration and sampling compartment(s) in the experimental designs that are theoretically adequate to quantitate the model parameters (14–16). Identifiability analysis requires the assumption of an infinite number of data points without error. However, the applicability of the predictions of identifiability analysis in situations where sampling is limited and experimental error is present needs to be tested. Thus, computer simulations and fittings of data without error are undertaken in the third step to investigate the limitation of this approach with respect to the finite number of samples possible in experimental situations. Finally, computer fitting of simulated data with 10% random error is utilized to evaluate the performance of the experimental designs found to be adequate by previous steps.

It should be understood that multiple physiological processes are represented by each of the rate constants in a compartmental model and not a single physiological process. In particular, the model in this investigation (Fig. 1) has been simplified by omitting the liver in order to demonstrate the experimental design strategy. However, a unique feature of this model is the inclusion of the intermittent gallbladder emptying as a separate process. For the purpose of this presentation, prior knowledge of the gallbladder emptying rate constant as a function of time is assumed to be available from using controlled emptying in an animal model with an engineered gallbladder or from quantitation in humans or animals using imaging techniques. Although such prior knowledge is, in theory, not required for the proposed model, it is necessary for more complex models.

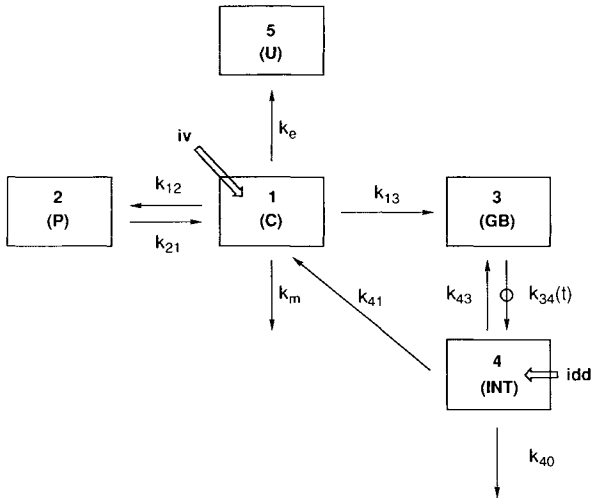

Fig. 1. An EHC model in which only the unchanged drug recycles. C is the central compartment, P is the peripheral compartment, U is the urinary compartment, GB is the gallbladder compartment, and INT is the intestinal compartment. The k symbols represent the intercompartmental transfer rate constants, and the gallbladder emptying rate constant $k_{34}(t)$ is a known function of time. Routes of administration are intravenous (iv) and intraduodenal (idd).

METHOD

Pharmacokinetic Model

A four-compartment model with one additional elimination compartment (Fig. 1) is proposed to depict the pharmacokinetics of most drugs that undergo EHC in unchanged form. The model includes distribution between central and peripheral compartments, elimination in urine, metabolism to noncycling metabolites, EHC of unchanged drug, and elimination from the intestine. EHC is represented in the model by three transfer processes: biliary excretion, gallbladder emptying, and intestinal reabsorption, with the corresponding rate constants being k_{13}, $k_{34}(t)$, and k_{41}, respectively. Since the gallbladder empties intermittently, the value of the first-order emptying rate constant varies with time. This rate constant, $k_{34}(t)$, which can be evaluated under appropriate experimental conditions (see Discussion), is assumed to be a known function of time. All other intercompartmental transfer processes are first-order with a time-invariant rate constant. The rate constant k_{43} represents first-pass hepatic extraction which involves multiple physiological processes, such as the transfer across the intestinal wall, hepatocellular uptake, transport to the gallbladder, etc.

Identifiability Analysis

Identifiability analysis was employed to determine the parameter(s) of the model in Fig. 1 that can theoretically be determined using several different experimental designs for the route(s) of administration and the choice of compartment(s) to be sampled (14–16). The Taylor-series approach is applicable to models with time-varying parameters, such as EHC models. Two routes of administration, intravenous (iv) and intraduodenal (idd), were considered, and two compartments were deemed accessible for sampling, central/plasma and gallbladder. Gallbladder sampling is possible in an animal model being developed in our laboratories (see Discussion). One route of elimination (urine) was sampled. Several combinations of route(s) of administration and sampling compartment(s) are thus possible and these different experimental designs were analyzed and the parameters that can be quantitated were determined. The experimental designs that would allow quantitation of all model parameters were then selected for further analysis.

Computer Simulation

NONLIN84 (17) was used on an IBM4361 computer for both simulation and fitting. Differential equations were written in FORTRAN for the model in Fig. 1 both for intravenous and for intraduodenal administration. Except for the cases in which only iv administration applies, both iv and idd equations were used simultaneously for simulation and fitting. Since gallbladder activity varies with time, a time-varying function was selected to describe the first-order

emptying rate constant, k_{34}. In every 3-hr period, the gallbladder emptying rate constant was defined to have a zero value in the first 2 hr and a value of 0.06 (min^{-1}) in the third hour. Therefore, two sets of differential equations were used in the FORTRAN subroutine to define the model, one for the time when the gallbladder emptying rate constant is zero and the other for the time when the rate constant is not zero. An IF statement was used to switch from one set to the other. The other parameter values were chosen to be in a reasonable range from a physiological standpoint and are listed in Tables II and VI as "true value." For a hypothetical compound which can be described by the chosen parameter values, about 80% of the drug molecules in the central compartment return during each cycle. The dose administered was chosen to be 100.0 mg for each route of administration.

Error-free concentration (amount)–time data for plasma, urine, and gallbladder were simulated for both intravenous and intraduodenal administration (six data sets). Data were then selected for subsequent fittings corresponding to sampling times at 10-min intervals in the first and third hour and at 30-min intervals in the second hour. The overall sampling frequency was 14 data points over a 3-hr gallbladder activity cycle. Then another six sets of data with 10% random error were generated by the addition of ±10% uniformly distributed random error to the corresponding simulated error-free data sets. A random number generator from PLOT79 (18) was used to obtain a set of random numbers from 0 to 1.0 and these were transformed linearly to a range of −10 to +10%. Only a portion of these simulated data was used when the particular experimental design did not require all six data sets. It should be understood that when data from the same compartment after the same route of administration were used in different experimental designs, the values were identical.

The timing of gallbladder emptying was chosen to be between 120 and 180 min during each gallbladder activity cycle, except for the case in which the timing of gallbladder emptying was investigated using a substitute period of 5–65 min chosen to coincide with the distribution process. Although a separate run of computer simulation and fitting was done for each schedule of gallbladder emptying activity, the sampling protocol was kept the same as described in the previous paragraph, and the percentage of random error associated with each specific data point in each run was kept the same.

Computer Fitting

Nonlinear least-squares fitting of the model in Fig. 1 to the simulated data sets (with and without random error) was completed using NONLIN84 (17) with the same FORTRAN subroutine previously described. The data sets from all the sampling compartments and all routes of administration for any particular experimental design were fit simultaneously using $1/y^2$ weighting with equal contribution of each data set for a particular route and sampling compartment (19). Microconstants (Fig. 1) and the volume of the central compartment (V_1) were iterated except for k_{34}, the known rate constant for gallbladder emptying. The initial estimates were arbitrarily chosen to be between 0.6 and 1.5 times the true values, unless the influence of initial estimates on fitting was being investigated. The weighted residual was calculated for all data points after the fitting of data with 10% random error by the formula (20)

$$(\text{observed value} - \text{predicted value}) \times \sqrt{\text{weight}}$$

and plotted against time as well as against the predicted value. The residual plots were compared among the different experimental designs. All computer fittings were repeated with six groups of data generated from the same simulated error-free data by adding ±10% error derived from six different randomizations.

Three variables were selected for evaluation with respect to their effect on computer fits of data with random error: the accuracy of the initial parameter estimates, sampling frequency, and timing of gallbladder emptying. The influence of initial estimates was investigated by using progressively poorer initial estimates in sequential runs, starting from either 0.6 or 1.5 times the true values and progressing in stages to either 0.005 or 200 times the true values. Sampling frequency was investigated by using data sets ranging from 14 data points per 3 hr to 3 data points per 3 hr. Timing of gallbladder emptying was investigated by varying this factor as described in the previous section. The effect of sampling frequency and the effect of timing of gallbladder emptying under the condition of having poor initial estimates were also studied using initial estimates that were 0.1 or 10 times the true values.

RESULTS

Identifiability Analysis

The results of the identifiability analysis are presented in Table I, which lists the pharmacokinetic constants that can be determined from each experimental design. For presentation purposes, the model parameters are classified into three types: distribution, cycling, and elimination. The distribution constants, V_1, k_{12}, and k_{21}, as well as k_{43} could be determined in all experimental designs that included the intravenous route. In order to determine the renal excretion constant k_e, it was necessary to sample the central and urinary compartments after iv or iv plus idd administration. All cycling constants could be quantitated under two situations: (a) sampling at least the central and gallbladder compartments after iv administration and (b) sampling at least the central compartment after separate iv and idd administration. Thus, three experimental designs are adequate in theory for determination of all pharmacokinetic constants of the model, including one volume of distribution and eight rate constants. These experimental designs are (i) sampling central (C), urinary (U), and gallbladder (GB) compartments after intravenous administration, denoted iv(C + U + GB); (ii) sampling C and U after two separate routes of administration, intravenous and intraduodenal, denoted iv & idd(C + U); and (iii) sampling C, U, and GB after two separate routes of administration, denoted iv & idd(C + U + GB). These three designs were then selected for the subsequent steps in the strategy for evaluating the performance of proposed experimental designs.

Table I. Identifiability Analysis for the Model in Fig. 1: Listing of Pharmacokinetic Constants that Can Be Determined from the Experimental Designs Indicated[a]

Compartment(s) sampled[b]	idd,[c] cycling[d]	Pharmacokinetic constant: iv[c] Distribution[d]	iv Cycling	iv Elimination[d]	iv & idd Distribution	iv & idd Cycling	iv & idd Elimination
C	—	V_1, k_{12}, k_{21}	k_{43}	—	V_1, k_{12}, k_{21}	k_{13}, k_{41}, k_{43}	k_{40}
C, U	—	V_1, k_{12}, k_{21}	k_{43}	k_e	V_1, k_{12}, k_{21}	k_{13}, k_{41}, k_{43}	k_e, k_m, k_{40}[e]
C, GB	—	V_1, k_{12}, k_{21}	k_{13}, k_{41}, k_{43}	k_{40}	V_1, k_{12}, k_{21}	k_{13}, k_{41}, k_{43}	k_{40}
C, U, GB	k_{43}	V_1, k_{12}, k_{21}	k_{13}, k_{41}, k_{43}	k_e, k_{40}, k_m[e]	V_1, k_{12}, k_{21}	k_{13}, k_{41}, k_{43}	k_e, k_m, k_{40}[e]

[a] The experimental designs include the route of administration and the compartment sampled.
[b] C, central; U, urinary; GB, gallbladder.
[c] Routes of administration are intraduodenal (idd) and intravenous (iv).
[d] Pharmacokinetic constants (Fig. 1) are classified as distribution, cycling, or elimination constants; k_{34} is a known function of time (see Discussion). Constants not listed cannot be determined.
[e] All constants can be determined from these experimental designs.

Computer Simulation and Fitting of Error-Free Data

The simulated plasma/central compartment concentration–time data, cumulative amount–time data in gallbladder bile, and cumulative urinary amount–time data after both intravenous and intraduodenal administration are presented in Figs. 2a and b for a hypothetical compound with high biliary excretion. The data cover a time period longer than two cycles of gallbladder activity. The cumulative amount–time curve for gallbladder bile after either iv or idd administration had a pattern of buildup for 2 hr and then declined for the third hour, which corresponded to the predefined gallbladder emptying activity cycle of resting for 2 hr and emptying for the third hour in every 3-hr period. The plasma concentration–time curve after iv administration is characterized by an initial decline in the first 2 hr due to distribution, elimination, and biliary excretion; in the third hour, the absorption process following gallbladder emptying triggered an increase in concentration. A similar pattern was observed in the plasma concentration–time curve after intraduodenal administration, except that there was an initial absorption phase in the first hour. The urinary compartment level increases with time over 3 hr since this is a cumulative elimination (sink) compartment. The gallbladder, plasma, and urine time profiles then repeat every 3 hr in a regular pattern.

Selected data points on the simulated curves, with an overall sampling frequency of 14 data points in every 3 hr, were used as hypothetical observations for computer fitting to estimate the parameter values. This was done to determine the adequacy of this sampling protocol under error-free conditions. Parameter estimates for the three experimental designs are listed in Table II. All the parameter estimates match closely with the true values used in the simulations and had small coefficients of variation, indicating an adequate sampling frequency. Therefore, all three experimental designs were investigated further using this sampling protocol and the introduction of 10% random error.

Computer Fitting of Data with Random Error

Parameter estimates obtained from linear least-squares fitting of one group of data with 10% random error (Fig. 2) are listed in Table III. Compared to the fits of data without

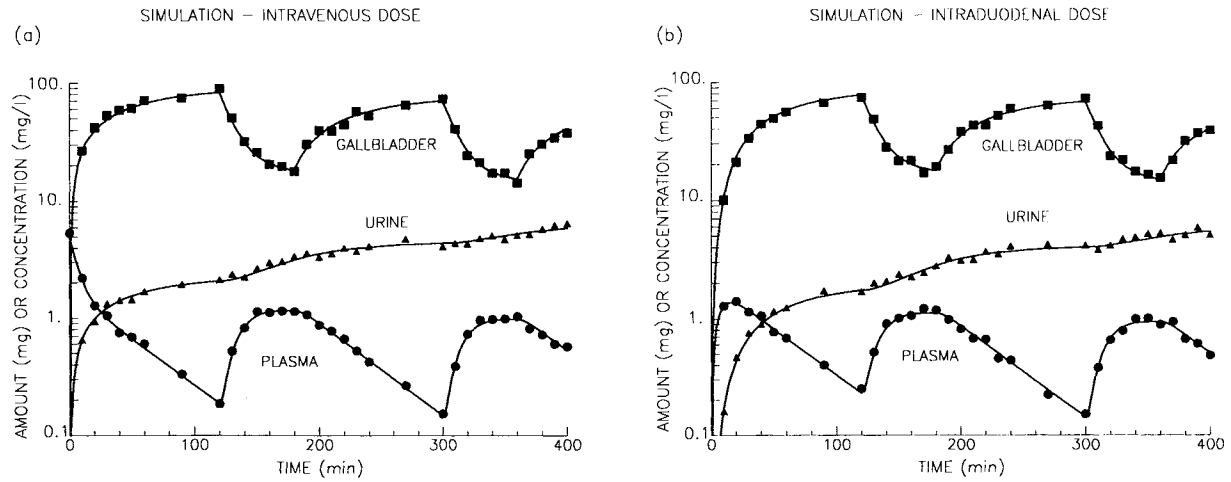

Fig. 2. Effect of 10% random error on simulated compartmental concentration (amount)–time data for plasma, urine, and gallbladder after a single iv or idd dose. The data points containing 10% random error are indicated by the symbols. The corresponding error-free data points on the simulated curves are not shown. The parameter values used in the simulation are listed in Table II.

Table II. Estimated Pharmacokinetic Constants from Nonlinear Least-Squares Fitting of Error-Free Data[a]

Parameter	True value	Least-squares estimate: iv (C + U + GB)[b]	iv & idd (C + U)	iv & idd (C + U + GB)
V_1 (L)	20.0	20.0 (0.071)[c]	20.0 (0.013)	20.0 (0.049)
k_{12} (min^{-1})	0.06	0.0600 (0.097)	0.0600 (0.045)	0.0600 (0.084)
k_{21} (min^{-1})	0.06	0.0600 (0.066)	0.0600 (0.028)	0.0600 (0.047)
k_{13} (min^{-1})	0.04	0.0400 (0.026)	0.0400 (0.015)	0.0400 (0.023)
k_{41} (min^{-1})	0.055	0.0550 (0.12)	0.0550 (0.0091)	0.0549 (0.042)
k_{43} (min^{-1})	0.005	0.00501 (0.26)	0.00500 (0.082)	0.00502 (0.073)
k_e (min^{-1})	0.001	0.00100 (0.034)	0.00100 (0.013)	0.00100 (0.024)
k_m (min^{-1})	0.003	0.00300 (0.10)	0.00300 (0.053)	0.00300 (0.038)
k_{40} (min^{-1})	0.003	0.00301 (0.15)	0.00300 (0.075)	0.300 (0.038)

[a] Pharmacokinetic constants and other abbreviations are defined in Fig. 1; k_{34} is a known function of time (see Discussion).
[b] Compartments sampled.
[c] Coefficient of variation (%).

Table III. Estimated Pharmacokinetic Constants from Nonlinear Least-Squares Fitting of Data with 10% Random Error[a]

Parameter	True value	Least-squares estimate: iv (C + U + GB)[b]	iv & idd (C + U)	iv & idd (C + U + GB)
V_1 (L)	20.0	23.8 (36)[c]	20.4 (8.7)	20.1 (25.)
k_{12} (min^{-1})	0.06	0.0294 (28)	0.0609 (30)	0.0341 (33)
k_{21} (min^{-1})	0.06	0.0299 (30)	0.0622 (19)	0.0393 (24)
k_{13} (min^{-1})	0.04	0.0361 (6.7)	0.0369 (9.7)	0.0379 (7.4)
k_{41} (min^{-1})	0.055	0.0779 (67)	0.0597 (6.1)	0.0428 (17)
k_{43} (min^{-1})	0.005	0.0000124 (86000)	0.00606 (47)	0.00561 (23)
k_e (min^{-1})	0.001	0.000837 (12)	0.000959 (8.5)	0.000953 (9.9)
k_m (min^{-1})	0.003	0.0000106 (13000)	0.00404 (24)	0.00273 (18)
k_{40} (min^{-1})	0.003	0.0117 (61)	0.00266 (58)	0.00257 (18)

[a] Pharmacokinetic constants and other abbreviations are defined in Fig. 1; k_{34} is a known function of time (see Discussion).
[b] Compartments sampled.
[c] Coefficient of variation (%).

error (Table II), parameter estimates from data with error do not match as closely with the true values, and the coefficients of variation are substantially greater than the corresponding coefficients in Table II. The parameter estimates for two experimental designs, iv & idd(C + U) and iv & idd (C + U + GB), have acceptable coefficients of variation (CV) using an arbitrary limit of acceptability of CV $\leq$100%. However, the third experimental design [iv(C + U + GB)] has an unacceptable coefficient of variation for two parameters, k_{43} and k_m. Thus, the data from this experimental design were fitted poorly. This was confirmed by the deviations of fitted curves from simulated curves (Fig. 3). When similar graphs of the other experimental designs were examined, all curves were virtually superimposable. The argument that the design of iv(C + U + GB) is poorer than the others was supported by the fits of the other five sets of data having different randomizations. A similar reliability problem with estimates of k_{43} was found in the fits of the other five data sets. The reliability of estimates of k_{40} and k_m depended on the specific randomization of error, as indicated by the incidence of CV >100% in two and three of the six groups of data sets, respectively.

In addition to the comparison of parameter estimates and the closeness of the fitted curves to the simulated curves, residual plots were used qualitatively to assess the goodness of fit. For each sampled compartment in every experimental design, the weighted residual was plotted against time, as well as against the predicted concentration or amount in the compartment. All residual plots from the experimental design of iv & idd(C + U) showed a random scattering of the data points, which suggests that there is neither concentration dependence nor time dependence in the fit from this experimental design. The experimental design of iv(C + U + GB), which was characterized by unacceptable coefficients of variation of fitted parameters (Table III) and deviations of fitted from simulated time profiles (Fig. 3), yielded a strong time-dependent pattern in the residual plot for the predicted plasma concentration. Similar residual plots for the third experimental design [iv & idd(C + U + GB)] had random scattering for all plots except urine after idd administration, which showed positive deviation for both independent variables, amount and time.

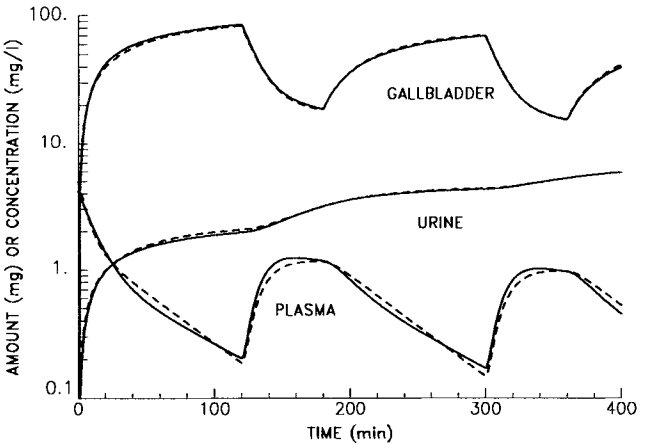

Fig. 3. Comparison of the simulated curves (dashed lines) and the fitted curves (solid lines) from the iv(C + U + GB) experimental design that yielded unreliable parameter estimates. The parameter values used in the simulation and those obtained from the computer fitting of simulated data with 10% random error are listed in Table III.

Other Experimental Design and Procedure Variables

The other important variables that influence nonlinear least-squares fitting are the initial estimates, sampling frequency, and timing of gallbladder emptying. Each of these variables was also investigated systematically.

The effect of varying the initial estimates for one of the randomizations on the nonlinear least-squares fits is summarized in Table IV for two experimental designs. It was found that the value of each parameter in column A for the design of iv & idd(C + U) was comparable to the true value and the CV (≤60%) was in the acceptable range, as defined previously. When initial estimates were extended from 0.1 or 10 times the true values (column A) to 0.02 or 50 times the true values (column B), only one parameter, k_{43}, had an unacceptable coefficient of variation. When the initial estimates were extended to 0.01 or 100 times the true values, the fitting failed for the design of iv & idd(C + U) (column C) but not for the design of iv & idd(C + U + GB) (column D). The latter finally failed when the initial estimates were 0.005 or 200 times the true values (column E). Thus, one design, iv & idd(C + U + GB), is more capable of withstanding poor initial estimates than the other, iv & idd(C + U), based on data in Table IV. In four of six randomizations, the data supported this finding with initial estimates as far as 0.01 or 100 times the true values. In the other two randomizations, neither design was superior. Most importantly, these results indicate that the computer fitting of the model to data from both experimental designs has good stability relative to the accuracy of initial estimates.

The effect of sampling frequency on the parameter estimates from computer fitting of one group of data is presented in Table V for the iv & idd(C + U) experimental design, and similar results were observed in fittings of the other five randomizations. Results were comparable for initial estimates that were 0.6 or 1.5 times the true values (Table V) and for initial estimates that were 0.1 or 10 times the true values. Generally, the coefficients of variation are larger for the low-sampling frequency case, 3 data points per 3 hr, than those for the high-sampling frequency, 14 data points per 3 hr (Table V). Coefficients of variation for 5 or 7 data points per 3 hr (data not shown) are comparable to the CVs for the 14 data points per 3 hr. When the sampling frequency was three points per 3 hr, the choice of alternative sampling times influenced the fit, with the reliability of certain parameters (k_{12}, k_{43}, and k_{40}) being dependent on the choice of sampling times (compare columns B and C in Table V). For the low-sampling frequency case the reliability of k_m and k_{40} estimates varied from one randomization to another. This was also true for k_{21}, but only for the wider initial estimates of 0.1 or 10 times the true value.

The effect of the timing of the gallbladder emptying (chosen to coincide or not to coincide with the distribution process) on the parameter estimates is presented in Table VI

Table IV. Effect of Initial Estimates on the Least-Squares Estimates for Fitting of Data with 10% Random Error[a]

		Least-squares estimates				
		iv & idd (C + U)[b]			iv & idd (C + U + GB)	
Parameter	True value	A[c]	B[d]	C[e]	D[f]	E[g]
V_1 (L)	20.0	21.0	21.1	1660	20.1	4270
		(8.7)[h]	(8.1)	(8300)	(25)	(13000)
k_{12} (min^{-1})	0.06	0.0544	0.0498	0.000543	0.0343	0.000307
		(30)	(29)	(88000)	(33)	(16000)
k_{21} (min^{-1})	0.06	0.0588	0.0543	0.000586	0.0395	0.000235
		(19)	(19)	(590000)	(24)	(20000)
k_{13} (min^{-1})	0.04	0.0356	0.0372	2.35	0.0380	3.19
		(9.4)	(9.2)	(530)	(7.5)	(2800)
k_{41} (min^{-1})	0.055	0.0600	0.0579	0.0409	0.0430	0.000215
		(6.2)	(6.0)	(35)	(17)	(3800)
k_{43} (min^{-1})	0.005	0.00626	0.000171	0.000129	0.00556	1.21
		(46)	(1500)	(12600)	(24)	(4100)
k_e (min^{-1})	0.001	0.000929	0.000921	0.0303	0.000954	0.201
		(8.1)	(7.8)	(520)	(9.9)	(2800)
k_m (min^{-1})	0.003	0.00397	0.00260	0.0000287	0.00273	0.0000190
		(24)	(38)	(590000)	(18)	(11000)
k_{40} (min^{-1})	0.003	0.00258	0.00515	0.000107	0.00257	2.55
		(60)	(30)	(7500)	(18)	(4100)

[a] Simulated data obtained after iv and idd dose are collected separately and fitted simultaneously.
[b] Compartments sampled.
[c] The initial estimates are either 10 × (true value) or 0.1 × (true value).
[d] The initial estimates are either 50 × (true value) or 0.02 × (true value).
[e] The initial estimates are either 100 × (true value) or 0.01 × (true value).
[f] The initial estimates are either 100 × (true value) or 0.01 × (true value).
[g] The initial estimates are either 200 × (true value) or 0.005 × (true value).
[h] Coefficient of variation (%).

Table V. Effect of Sampling Frequency on Parameter Estimates from Nonlinear Least-Squares Fitting of Data[a] with 10% Random Error

Parameter	True value	Least-squares estimate A[b]	B[c]	C[d]
V_1 (L)	20.0	20.4	18.9	15.9
		(8.7)[e]	(26)	(17)
k_{12} (min^{-1})	0.06	0.0609	0.0795	0.0756
		(30)	(89)	(14)
k_{21} (min^{-1})	0.06	0.0622	0.0877	0.0528
		(19)	(52)	(25)
k_{13} (min^{-1})	0.04	0.0369	0.0373	0.0501
		(9.7)	(25)	(20)
k_{41} (min^{-1})	0.055	0.0597	0.0569	0.0453
		(6.1)	(5.7)	(14)
k_{43} (min^{-1})	0.005	0.00606	0.00780	0.000138
		(47)	(70)	(2800)
k_e (min^{-1})	0.001	0.000959	0.00101	0.00123
		(8.5)	(22)	(16)
k_m (min^{-1})	0.003	0.00404	0.00541	0.00435
		(24)	(45)	(65)
k_{40} (min^{-1})	0.003	0.00266	0.00200	0.00242
		(58)	(150)	(88)

[a] Data from simulated plasma concentration–time curves and urinary amount–time curves after iv and idd dose are collected separately and fitted simultaneously.
[b] The sampling frequency is 14 time points per 3 hr.
[c] The sampling frequency is three time points per 3 hr. The sampling times are 10, 90, and 170 min after the beginning of each cycle.
[d] The sampling frequency is three time points per 3 hr. The sampling times are 40, 90, and 140 min after the beginning of each cycle.
[e] Coefficient of variation (%).

Table VI. Effect of the Timing of Gallbladder Emptying on the Parameter Estimates Obtained by Fitting Data with 10% Random Error[a]

Parameter	True value	Least-squares estimate: iv & idd (C + U)[b] 5–65 min[c]	iv & idd (C + U)[b] 120–180 min[d]	iv & idd (C + U + GB) 5–65 min	iv & idd (C + U + GB) 120–180 min
V_1 (L)	20.0	19.8	20.4	19.7	20.1
		(13)[e]	(8.7)	(36)	(25)
k_{12} (min^{-1})	0.06	0.0702	0.0609	0.0306	0.0341
		(50)	(30)	(50)	(33)
k_{21} (min^{-1})	0.06	0.0746	0.0622	0.0501	0.0393
		(29)	(19)	(26)	(24)
k_{13} (min^{-1})	0.04	0.0376	0.0369	0.0359	0.0379
		(16)	(9.7)	(13)	(7.4)
k_{41} (min^{-1})	0.055	0.0564	0.0597	0.0287	0.0428
		(9.3)	(6.1)	(15)	(17)
k_{43} (min^{-1})	0.005	0.000273	0.00606	0.00770	0.00561
		(390)	(47)	(10)	(23)
k_e (min^{-1})	0.001	0.000977	0.000959	0.000948	0.000953
		(13)	(8.2)	(15)	(9.9)
k_m (min^{-1})	0.003	0.00421	0.00404	0.00263	0.00273
		(27)	(24)	(19)	(18)
k_{40} (min^{-1})	0.003	0.00297	0.00266	0.00236	0.00257
		(55)	(58)	(13)	(18)

[a] Pharmacokinetic constants and other abbreviations are defined in Fig. 1; k_{34} is a known function of time (see Discussion).
[b] Compartments sampled.
[c] The gallbladder empties between 5 and 65 min in every 3-hr period.
[d] The gallbladder empties between 120 and 180 min in every 3-hr period.
[e] Coefficient of variation (%).

for two experimental designs. The parameter estimates and the coefficients of variation obtained from fitting data with two different gallbladder emptying schedules are quite comparable for the experimental design iv & idd(C + U + GB). However, there is an unacceptable coefficient of variation for the parameter k_{43} in the other experimental design, iv & idd(C + U), for the 5- to 65-min time period that coincides with the distribution process (Table VI). The same results were observed in fitting all six groups of data provided with two different sets of initial estimates, one being 0.6 or 1.5 times the true values and the other being 0.1 or 10 times the true values.

DISCUSSION

Thus far, the reliability of methods for quantitating complex pharmacokinetic models has not been challenged. The typical study of a complex phenomenon, such as EHC, involves fitting of plasma concentration–time data for a drug or drug–conjugate pair to a hypothetical model that is often poorly validated. The complexity of the model is not adequately considered in the choice of sampling compartments, routes of administration, or sampling times. The resulting pharmacokinetic constants from such a fit have unknown reliability. In addition, pharmacokinetic data resulting from a model that contains an intermittent process, such as gallbladder emptying, have not been analyzed successfully. The strength of the strategy used in this investigation lies in the use of model, data, and data analysis that closely correspond to the *in vivo* experimental situation in order to rule out experimental designs that are clearly inadequate. This includes the incorporation of intermittent gallbladder emptying as an integral component of the EHC process. An especially important contribution is the incorporation of the step in which simulated data with random error are fit, and the result assessed by comparing with the "true values" of the pharmacokinetic constants used to obtain the simulated data and by evaluating coefficients of variation and residual plots.

The model for drugs undergoing EHC in unchanged form was selected to include gallbladder, intestine, peripheral, and central compartments, thus including the important kinetic steps in the EHC process. Gallbladder emptying was defined as a known intermittent process, with essentially complete emptying within the 1-hr emptying time. (The $t_{1/2}$ for gallbladder emptying in humans is 10–30 min.) Prior knowledge of the gallbladder emptying rate constant was found to be necessary for evaluating more complex models from previous identifiability analysis studies (unpublished). Such prior knowledge is clearly possible in an experimental animal model with controlled gallbladder emptying, as is being developed in our laboratories, and is not impossible in animals and humans because imaging techniques can be used to monitor gallbladder volume as a function of time (21–23).

Finally, it is necessary to exclude the potentially competing intermittent process of gastric emptying by idd administration of drugs (rather than oral) and by excluding weak bases that could be secreted into the stomach and emptied intermittently. This could be accomplished by administering the dose via a catheter positioned in the duodenum.

There are two common methods used for identifiability analysis, the Laplace transform method (14) and the Taylor-series method (14–16). The former is limited to time-invariant, linear model systems. The Taylor-series approach, which was used in this investigation, is applicable to models with nonlinear or time-varying kinetic processes (such as gallbladder emptying).

Identifiability analysis results is a list of rate constants that can be determined for a particular model and a particular experimental design for the sampling compartment(s) and route(s) of administration. However, it is important to realize that identifiability analysis assumes an infinite number of sampling times and is, thus, a "theoretical" design. The theoretically necessary experimental designs for quantitation of the EHC model requires either (i) sampling the gallbladder bile, plasma, and urine after iv administration, (ii) iv and idd administration with sampling of plasma and urine, and (iii) iv and idd administration with sampling of plasma, urine, and bile (Table I). These results indicate that the usual designs for pharmacokinetic studies are not adequate for investigating EHC since gallbladder bile is never sampled and idd administration is not utilized. One does encounter pharmacokinetic studies in which both oral and iv administration are used and plasma and urine are sampled, but this design permits the intermittent process of gastric emptying of the oral dose to superimpose on the intermittent gallbladder emptying, whereas this does not occur with idd dosing.

Among the three experimental designs found to be adequate by the identifiability analysis, iv(C + U + GB) was a poor design when data with random error were fit, as indicated by the unacceptable CV for two parameters (Table III), the appreciable deviation of the fitted time profiles from the simulated ones (Fig. 3), and the time dependence of the weighted residual. When the remaining two experimental designs, iv and idd(C + U) and iv & idd(C + U + GB), were compared, it was found that the fit for data with random error from the latter is more capable of withstanding poor initial estimates and poor timing of the gallbladder emptying (simultaneous with distribution). The major reason accounting for this observation is that k_{43} is better defined in the presence of data from the gallbladder, as suggested by the fact that k_{43} easily lost the reliability of its estimated value when the initial parameter estimates were poor (Table IV) and when the timing of gallbladder emptying was chosen to make two exponential processes overlapping with each other, i.e., distribution and absorption (Table VI).

Whether applying the presented strategy to studies in experimental animals with controlled gallbladder activity or to humans or animals with physiological gallbladder function, preliminary experiments are necessary to determine and verify the model structure, to optimize the sampling protocol, and to obtain initial estimates for the model parameters. Although the specific preliminary experiments are unique to each experimental situation, potential examples include (i) interrupting EHC to determine the number of peripheral distribution compartments and to obtain initial estimates of the distribution rate constants from iv plasma level–time data, (ii) collection of bile and urine, as well as plasma, after iv administration in a surgically prepared experimental animal to obtain initial estimates of urinary and biliary excretion rate constants, as well as to assess the need for a gallbladder compartment, and (iii) iv & idd administration in a bile duct-cannulated animal to assess the rate constant for absorption after idd administration. The closer the initial estimates of pharmacokinetic parameters are to the real *in vivo* values, the greater the likelihood that the application of the strategy in this investigation to *in vivo* pharmacokinetic studies of complex models will result in reliable estimates of pharmacokinetic constants. The rigorous design of preliminary experiments offers another valuable advantage of defining the compartmental model more accurately and thereby minimizing the problem of model indistinguishability in compartmental analysis (24).

Models more complex than the one presented here can be used for closer approximation of the physiology of EHC. However, the analysis would be more difficult mathematically and the practical limitations on sampling compartments, sampling times, and routes of administration can make it impossible to determine all the parameters in the more complex model. Nevertheless, the quantitation of some of the model parameters can still be valuable even though the model is too complex to be evaluated completely. Further investigations on the potential of modeling EHC in humans requires much additional theoretical and experimental work, and the outcome for success is not clear. However, we should not underestimate the potential that future advances in technology may have for measuring drug concentrations in various parts of the body *in vivo*.

In conclusion, an experimental design strategy has been presented for the investigation of complex pharmacokinetic models, and it has been demonstrated using a model for EHC of unchanged drug. The problem of developing a rigorous design for investigating EHC was addressed in this study for the first time. The results of the present investigation indicate that pharmacokinetic methodologies developed previously are inadequate for evaluating EHC or other complex pharmacokinetic phenomena, because of inadequate sampling compartments, inadequate sampling times, and insufficient distinction between idd and oral administration. Since the fitting of drug level–time data from multiple compartments is likely to be subject to local minima and sensitive to sampling frequency and the accuracy of initial estimates, it is important to evaluate the performance of the computer fitting for different experimental designs with a set of simulated data that is as close to the proposed experimental situation as possible. Such simulated data (with a random error that approximates the expected experimental error) could be obtained from the preliminary experiments described previously.

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