compute Q_B and I, respectively. Values of E were simply obtained as the differences between computed values of Q_B and their corresponding values of A_t.

SUMMARY AND CONCLUSIONS

An approach is suggested for the development of "optimized *in vitro* drug-release tests" having a maximum capability of predicting *in vivo* drug bioavailability from dosage forms as a function of formulation factors. Such tests, appropriately applied with caution, could minimize the extent of human testing required to develop drug products with optimal *in vivo* drug-release characteristics. It is further demonstrated that with appropriate drugs for which a continuously graded pharmacological response intensity is observable, the results of optimized drug-release testing can be utilized to compute the time course of pharmacological activity.

The development of such a capability represents the ultimate in *in vitro* drug-release testing that can be sought. However, because of practical considerations such as the large magnitudes of intersubject and intrasubject variation commonly observed with *in vivo* data and the large amount of experimentation that may be necessary to develop optimized tests providing *in vivo* correlations within acceptable statistical limits, *in vitro* drug-release tests may remain constrained in their applicability. An intrinsic limitation is their applicability only to those cases where the systemic availability of the drug is rate limited by its release from the dosage form (3). Obviously, the development and subsequent application of *in vitro* drug-release tests should be undertaken with an awareness of such limitations. However, the inclusion of an appropriate membrane (13) as a permselective barrier in the *in vitro* drug-release testing apparatus could ultimately allow this latter serious limitation to be surmounted.

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Problems Associated with Analysis of Pharmacokinetic Models

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Abstract □ When a pharmacokinetic model is fitted to blood levels of a drug, the estimates of the pharmacokinetic parameters are likely to be subject to considerable error. These errors are probably unimportant as long as the model is used only to predict blood levels. However, when the parameters are used to predict other features of the system (*e.g.*, tissue drug levels), considerable errors in prediction may result. An example based on simulated data is devised to illustrate this possibility. It is further suggested that if the pharmacokinetic parameters are derived for the purpose of predicting the blood levels for different regimens or formulations of the drug—such as multiple-dose regimens or sustained-release capsules—this end can be met more expeditiously by using purely empirical techniques.

Keyphrases □ Pharmacokinetic models—analysis problems □ Blood levels, drugs—parameter predictions □ Lithium carbonate pharmacokinetics—two-compartment model □ Tissue drug levels—prediction errors

Over the past few years, it has become fashionable to conduct studies in which blood levels of a drug are determined at various times after administration; the resulting data then are fitted to a pharmacokinetic model. An example is the two-compartment model with oral administration, in which the usual assumptions of first-order kinetics lead to the expression:

$$C(t) = \frac{k_a D}{V_c}\left[\frac{(\alpha - k_{21})}{(\alpha - \beta)(k_a - \alpha)}e^{-\alpha t} + \frac{(k_{21} - \beta)}{(\alpha - \beta)(k_a - \beta)}e^{-\beta t} - \frac{(k_a - k_{21})}{(k_a - \alpha)(k_a - \beta)}e^{-k_a t}\right] \quad \text{(Eq. 1)}$$

for the drug concentration in the central (plasma) compartment. The constants in this expression are the rate constants k_a (gut to plasma compartment), k_{12} and k_{21} (plasma compartment to tissue compartment and vice versa), k_e (elimination from plasma compartment), and D/V_c (amount of drug absorbed divided by the volume of distribution of the plasma compartment). The parameters α and β are defined by the relations $\alpha\beta = k_e k_{21}$ and $\alpha + \beta = k_e + k_{12} + k_{21}$. The usual problem is to find the values for these five constants which best fit Eq. 1 to a set of empirical data. This is an exercise in nonlinear least squares, usually carried out with a computer program which utilizes iterative schemes for finding the best fit.

It is well known, however, that the estimates of the parameters obtained are often extremely unreliable, especially if the values of any pair of the constants k_a, α, and β in the three exponential terms are at all close together (1). The purposes of this paper are to sound a warning note in this connection and to illustrate the problem by a simulation of actual blood level data. The unreliability of the estimates is related to the fact that, in many practical cases, wide variations in the estimates of the parameters give rise to only minute changes in the goodness of fit as measured by the residual sum of squares in the nonlinear estimation. In other words, there is a wide range of values for the constants k_a, α, β, k_{21}, and D/V_c for which essentially equally good fits of the model to the data are available. This is characterized in statistical terms by the fact that the confidence intervals associated with the estimates of the parameters are very wide. For example, if the least-squares estimate of k_a is found to be 3.85 hr.$^{-1}$, the 95% confidence interval might well be of the order of 0.90–6.80 hr.$^{-1}$. Extremely wide confidence intervals are likely to be the rule rather than the exception, except where the data accurately conform to the model under consideration and are obtained to an extremely high degree of precision.

Clearly, with the wide confidence intervals typically encountered, one cannot place great faith in the estimates of rate constants *per se*—at least not without a considerable amount of replication of the experiment. It can then be asked: To what practical purposes will the estimates of the rate constants be put? One answer is that they can be used to predict drug concentrations in the blood (or in other parts of the system) arising from given dosage regimens or new experimental formulations. This paper discusses this prediction problem and makes two main points:

1. While estimates of pharmacokinetic parameters from blood drug levels may be subject to considerable error, they usually allow reasonably accurate predictions to be made as long as these predictions are confined to blood levels. However, when one wishes to make such predictions (*e.g.*, for multiple-dose regimens or sustained-release formulations), this can be achieved more expeditiously by employing elementary superposition (or overlaying) techniques on the experimental blood level values themselves.

2. If the pharmacokinetic parameters are used to predict drug concentrations in some part of the system other than the blood compartment, considerable errors in prediction are possible. Examples are constructed to demonstrate this possibility.

As an example of prediction of blood levels of a drug, suppose that an experiment with a certain formulation of a drug gives hourly values $x_1 \ldots x_{12}$ of the blood drug levels from 0 to 12 hr. and that thereafter a few additional values are obtained (*e.g.*, at 18 and 24 hr.). If, during the first 12 hr. the samples are not obtained exactly on an hourly schedule, any missing hourly values are simply interpolated. To utilize simple superposition techniques, the hourly blood levels from 12 hr. on must be known; that is, x_i (for all integer values of $i > 12$) are needed. In practice, this is often not difficult to accomplish since the final exponential decay of the blood levels can frequently be modeled very easily. The values of x_t ($i > 12$) are then given by $Be^{-\beta(i-12)}$, where B and β are constants obtained by fitting the final exponential decay of the blood level curve. The values $x_1 \ldots x_{12}$ and the function $Be^{-\beta(i-12)}$ now become the input for a computer program which generates the blood levels from a given dosage regimen. For example, if the same formulation of the drug is given at 12-hr. intervals starting at 0 hr., then by superposition the predicted blood level at 27 hr. after the start of dosing (3 hr. after the third dose) is: $x_3 + Be^{-\beta(15-12)} + Be^{-\beta(27-12)}$, where the contribution x_3 is due to the dose at 24 hr., the second term is due to the dose at 12 hr., and the third term is due to the initial dose.

Steady-state blood levels, after infinite dosing, are easily obtained in a similar manner. For example, the predicted steady-state blood level at 3 hr. after a dose is taken is simply:

$$x_3 + Be^{-\beta(15-12)} + Be^{-\beta(27-12)} + Be^{-\beta(39-12)} \ldots = x_3 + Be^{-3\beta}/(1 - e^{-12\beta}) \quad \text{(Eq. 2)}$$

Most examples of predictions of blood levels from specified dosage regimens or experimental sustained-release formulations can be handled in this way. The only principles involved are those of superposition and

Table I—Simulated and Predicted Blood Level Data

Hours	Exact Blood Levels Generated by Two-Compartment Model	Exact Blood Levels with Added Noise	Blood Levels Predicted by Least-Squares Fit of Pharmacokinetic Parameters	Percentage Error in Predictions (Relative to Exact Levels)
0.25	0.18040	0.17489	0.17720	−1.8
0.5	0.25175	0.25877	0.25101	−0.3
0.75	0.26869	0.26085	0.27001	0.5
1.0	0.26015	0.26474	0.26200	0.7
1.5	0.21919	0.22221	0.21951	0.1
2	0.17869	0.17596	0.17770	−0.6
3	0.12672	0.12859	0.12788	0.9
6	0.08240	0.08374	0.09182	11.4
9	0.06991	0.08632	0.07846	12.2
12	0.06074	0.07290	0.06737	10.9
15	0.05287	0.05871	0.05784	9.4
18	0.04603	0.04384	0.04967	7.9
21	0.04007	0.04399	0.04265	6.4
24	0.03489	0.03410	0.03662	5.0

Table II—Least-Squares Estimates of Drug Concentration Parameters[a]

	Actual Values of Parameters	Least-Squares Estimates of Parameters	Approximate 95% Confidence Intervals	
k_a	2.389	1.774	−0.54	4.09
α	0.914	1.387	−0.61	3.38
β	0.0462	0.0508	0.036	0.065
k_{21}	0.250	0.333	0.211	0.455
D/V_c	0.441	0.570	−0.13	1.27
Residual sum of squares	0.00066	0.00037		

[a] Rate constants are in hr.$^{-1}$; D/V_c is in meq./l.

of scaling the blood levels by the appropriate factor whenever the dose differs from that on which the experimental data were obtained. This empirical "overlaying" principle depends only on the linearity of the transfers from one compartment to another, an assumption that is freely made in most pharmacokinetic analysis. Despite its simplicity, therefore, the technique is really a more sophisticated method than compartmental analysis for handling multiple-dose regimens and sustained-release formulations, since it is valid under less restrictive conditions than are required for any given compartmental analysis.

The second of the two points made earlier concerned the prediction of drug levels in parts of the system other than the blood compartment. In the two-compartment model, the obvious factor of interest that cannot be obtained without pharmacokinetic analysis is the concentration of drug in the tissue compartment. Gibaldi (2) made an interesting comparison of rapid intravenous injection and intravenous infusion by examining the differences in tissue drug levels that they generate. The warning referred to earlier is that the estimates of the pharmacokinetic parameters, although giving a good fit to the data, may be subject to considerable error. This error generally will not be of any consequence as long as the parameters are used only to predict future blood levels over the same time span for which data were obtained. However, if the parameters are used to predict any characteristic of the system other than blood levels, the errors in the parameter estimates may result in appreciable errors in these predictions. The following example was devised to demonstrate this possibility.

Table III—Exact and Predicted Tissue Drug Levels

Hours	Exact Tissue Drug Levels	Predicted Tissue Drug Levels	Percentage Error of Predicted over Exact
0.25	0.01358	0.02169	59.7
0.50	0.04198	0.06727	60.3
0.75	0.07398	0.11842	60.1
1.0	0.10435	0.16625	59.3
1.5	0.15321	0.24002	56.7
2	0.18552	0.28433	53.3
3	0.21562	0.31619	46.6
6	0.21074	0.28881	37.0
9	0.18496	0.24831	34.3
12	0.16112	0.21322	32.3
15	0.14027	0.18308	30.5
18	0.12212	0.15720	28.7
21	0.10631	0.13498	27.0
24	0.09255	0.11590	25.2

EXPERIMENTS ON SIMULATED DATA

From experiments with lithium carbonate administered as a solution in human volunteers, the pharmacokinetic parameters for a two-compartment model were fitted. Typical estimates in one volunteer (who was administered 300 mg. of lithium carbonate in solution) were $k_a = 2.389$ hr.$^{-1}$, $\alpha = 0.914$ hr.$^{-1}$, $\beta = 0.0462$ hr.$^{-1}$, $k_{21} = 0.250$ hr.$^{-1}$, and $D/V_c = 0.441$ meq./l. These parameters then were used to generate artificial data at various times based on the expression for drug concentration in the plasma compartment. These data are given in Column 1 of Table I. If one attempted to fit these data to the two-compartment model, a perfect fit would result, with the residual sum of squares essentially equal to zero and the parameter estimates identical to those used to generate the data. A low level of random noise was added to the blood levels in the first column, using random normal deviates with $\sigma = 0.01$. The second column shows the resulting set of data. These concentrations correspond to the sort of data one might obtain in an actual experiment if the system behaved as a perfect two-compartment model but with a small random error being introduced in the assay procedure for determining blood drug levels. The error σ is assumed to be the same for each data point. In practice, the error σ could be proportional to the actual drug concentration. This case is not treated here, although similar examples could easily be constructed for it.

The data in the second column were then fitted by least squares to the two-compartment model. This is the problem of estimating the five parameters (k_a, α, β, k_{21}, and D/V_c) in Eq. 1. The nonlinear least-squares program used is an adaptation of one written at the University of Wisconsin Computing Center and is based on the algorithm of Marquardt (3). Convergence was assumed complete with the iteration for which the relative change in the error sum of squares was less than 10^{-4}. The least-squares fit for the parameters is given in Table II, together with the actual values of the parameters. The 95% confidence intervals given are the simple (univariate) confidence intervals for each parameter individually.

Thus, the parameter values as estimated are appreciably different from the true values (note particularly a 26% error in k_a and a 50% error in α). However, the considerable width of the approximate 95% confidence intervals given in the last column should immediately warn one not to place too much faith in the actual values of the estimates. This simulation was performed with several sets of random noise. In a number of cases, the quite small amount of noise added was sufficient to disguise the two-compartment nature of the model, and the best least-squares fit reduced the model to the one-compartment case in which k_a and α were essentially equal. The example given here is typical of one of the "better" examples in which a reasonable distinction between k_a and α was possible.

It was suggested earlier that, even though the least-squares estimates of the pharmacokinetic parameters might be considerably in error, they will probably enable reasonably accurate predictions of blood levels to be made. That such, in fact, is the case can be seen by referring back to Columns 3 and 4 of Table I: the predicted blood levels and their percentage error over the correct levels, respectively. The error is, in general, less than 1% over the first 3 hr., when the levels of drug in the blood are appreciable. Thereafter, the proportional error becomes higher, although this is in large part due to the fact that the blood levels are becoming rapidly smaller and errors tend to be *proportionately* higher. Contrast this situation with the prediction of drug levels in the tissue compartment given by the equation:

$$T(t) = \frac{k_a k_{12} D}{V_T} \times \left[\frac{e^{-kat}}{(k_a - \alpha)(k_a - \beta)} - \frac{e^{-\alpha t}}{(\alpha - \beta)(k_a - \alpha)} + \frac{e^{-\beta t}}{(k_a - \beta)(\alpha - \beta)}\right] \quad \text{(Eq. 3)}$$

Table III shows the exact concentrations together with the predicted concentrations and the percentage error of predicted over exact. For convenience, the volume of distribution, V_T, for the tissue compartment has been assumed to be equal to that for the

Table IV—Exact and Predicted Blood and Tissue Drug Levels

Hours	Exact Blood Drug Levels	Predicted Blood Drug Levels	Percentage Error in Predictions (Relative to Exact)	Exact Tissue Drug Levels	Predicted Tissue Drug Levels	Percentage Error in Predictions (Relative to Exact)
0.25	0.13476	0.13202	−2.0	0.00449	0.00484	7.8
0.5	0.19500	0.19307	−1.0	0.01438	0.01555	8.1
0.75	0.21680	0.21596	−0.4	0.02632	0.02848	8.2
1.0	0.21919	0.21887	−0.1	0.03860	0.04171	8.1
1.5	0.20104	0.20012	−0.5	0.06136	0.06578	7.2
2	0.17570	0.17339	−1.3	0.08030	0.08505	5.9
3	0.13221	0.12830	−3.0	0.10725	0.11031	2.9
6	0.06899	0.06830	−1.0	0.13670	0.12909	−5.6
9	0.04900	0.05069	3.4	0.13612	0.12042	−11.5
12	0.04111	0.04266	3.8	0.12800	0.10748	−16.0
15	0.03671	0.03715	1.2	0.11847	0.09497	−19.8
18	0.03346	0.03264	−2.5	0.10914	0.08371	−23.3
21	0.03068	0.02873	−6.4	0.10040	0.07375	−26.5
24	0.02819	0.02530	−10.3	0.09233	0.06496	−29.6

central compartment, V_c. This has no significance, however, since the present concern is only with proportional errors.

The predicted parameters consistently overestimate the tissue drug levels, starting with a nearly 60% proportional error and decreasing to 25% by 24 hr. At the peak tissue levels (say from 2 to 9 hr.), the error ranges from about 50 to 35%. The consistency of the overestimation would lead to a substantial overestimation of the total amount of drug flowing into the tissue compartment.

A similar simulation is now presented based on the parameters: $k_a = 2.529\ \text{hr.}^{-1}$, $\alpha = 0.432\ \text{hr.}^{-1}$, $\beta = 0.028\ \text{hr.}^{-1}$, $k_{21} = 0.101\ \text{hr.}^{-1}$, and $D/V_c = 0.302$ meq./l. Again, the constants were suggested by experiments with lithium carbonate solution. After addition of random noise ($\sigma = 0.01$) to the blood drug levels, the least-squares estimates of the parameters were: $k_a = 2.327\ \text{hr.}^{-1}$, $\alpha = 0.5198\ \text{hr.}^{-1}$, $\beta = 0.0423\ \text{hr.}^{-1}$, $k_{21} = 0.1457\ \text{hr.}^{-1}$, and $D/V_c = 0.3166$. In contrast with the earlier simulation, k_a, α, and D/V_c are estimated reasonably accurately but there are larger errors in β and k_{21}. Table IV gives the exact blood and tissue drug levels and the predicted blood and tissue drug levels based on the least-squares estimators. In this case, the estimated parameters give predictions for the blood levels that are all within 4% of the true blood levels right up to the last two time intervals (21 and 24 hr.). By contrast, the predicted tissue drug levels show considerably greater errors. They are neither so consistently nor so grossly in error as the predictions of the previous simulation, but, nevertheless, they still give a distorted picture of the true tissue drug levels. Reasonably accurate predictions (say within 5%) are achieved only somewhere between 2 and 6 hr. Before 3 hr., the predictions overestimate drug tissue levels; after 6 hr., there is an ever-increasing underestimation.

In conclusion, two points should be made. First, these two examples of the considerable disparity between exact tissue drug levels and predicted levels based on least-squares estimates of the pharmacokinetic parameters are in no sense unusual. Many other similar examples have been constructed. Second, similar experiments with simulated data have confirmed the assertion made earlier that predictions of blood drug levels for sustained-release capsules or multiple-dose regimens can be made more accurately by using simple superposition techniques than by using estimations of pharmacokinetic parameters.

DISCUSSION

Certain problems involved in the fitting of pharmacokinetic models to blood level data are sometimes glossed over in published studies. The problems are statistical and are inherent in the attempt to fit data to functions consisting of sums of exponential terms. Confidence intervals for least-squares estimates of parameters are frequently wide, and there may be substantial errors in the estimates. A simple example was constructed to show the erroneous conclusions that can be drawn from these least-squares estimates when they are used to make predictions *other* than blood levels. It is further noted that when blood level prediction is sought, as for example in designing drug formulations, empirical methods should give predictions that are as good or better than those based on the parameter estimates derived from compartment models.

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