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Analysis of Animal Pharmacokinetic Data: Performance of the One Point Per Animal Design

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A simulation study was carried out to determine the impact of various design factors on the accuracy and precision with which population pharmacokinetic parameters are estimated in preclinical pharmacokinetic studies. A drug given by intravenous bolus injection and having monoexponential disposition characteristics was assumed. The factors investigated were (i) number of animals sampled at specified times with one observation taken per animal, (ii) error in observed concentration measurements, and (iii) doubling the number of observations per animal while varying the number of animals. Data were analyzed with the NONMEM program, and the least number of animals per time point (where each animal supplied one concentration–time point) required for accurate and precise parameter estimation was determined. The one observation per animal design yielded biased and imprecise estimates of variability, and residual variability could not be estimated. Increasing the error in the concentration measurement led to a significant deterioration in the accuracy and precision with which variability was estimated. Obtaining a second sample from each animal practically eliminated bias and facilitated the partitioning of interanimal variability and residual intraanimal variability, by introducing information about the latter. Doubling the total number of observations per animal required using half (i.e., 50) the total number of animals required for accurate and precise parameter estimation with the one sample per animal design.

KEY WORDS: population pharmacokinetics; preclinical; parameter estimation; sample size; variability; one sample per animal.

INTRODUCTION

Pharmacology and toxicology studies are frequently performed in which a drug is administered to a homogeneous group of small animals (rats and

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mice). Subgroups of the animals are sacrificed at specified times and their plasma assayed for the drug. The data for each time point are averaged and these values are used to estimate the pharmacokinetic parameters (1–4). This method gives estimates of average parameter values but gives no information about their distributions within the sample of animals studied.

In this paper, we report the results of simulation studies carried out to investigate the ability to obtain estimates of interanimal variability from traditional small animal pharmacokinetic data using the NONMEM (5) program.

A drug having the characteristics of avicin (6), a cytotoxic agent, was used to investigate the effects of a number of important experimental design features on the estimation of population pharmacokinetic parameters. These features were (i) varying the number of animals sampled per time point, (ii) changing the error in the observed concentration measurements, and (iii) varying the total number of samples (i.e., doubling the number of samples per animal with or without halving the number of animals).

METHODS

Pharmacostatistical Models

A monoexponential pharmacokinetic model with an intravenous bolus dose input was specified. The concentration at time t after drug administration was given by

$$C_j^* = (D/V_j)\exp(-CL_j/V_j \cdot t) \qquad (1)$$

where C_j^* is the model predicted concentration in the jth animal. The parameters for the jth animal are clearance, CL_j, and volume of distribution, V_j, and these were sampled from normal distributions, $N(CL, \sigma_{\mathrm{CL}}^2)$ and $N(V, \sigma_{\mathrm{V}}^2)$, respectively. For the purpose of the study the dose (3 mg) was assumed constant.

Each concentration was subject to a proportional error so that the observed concentration $C_j(t)$ was, therefore, given by

$$C_j(t) = C_j^*(t)\exp(\varepsilon_j) \qquad (2)$$

where the ε was assumed to be normally distributed with zero mean and variance σ^2. Interanimal variability in CL and V was modeled as follows:

$$CL_j = CL + \eta_j^{\mathrm{CL}};\ \eta_j^{\mathrm{CL}} \sim N(0, \sigma_{\mathrm{CL}}^2) \qquad (3)$$

$$V_j = V + \eta_j^{\mathrm{V}};\ \eta_j^{\mathrm{V}} \sim N(0, \sigma_{\mathrm{V}}^2) \qquad (4)$$

where η_j^{CL} and η_j^{V} are random individual deviations of CL_j and V_j from the corresponding mean population values (CL and V, respectively). The ηs are

independent identically distributed random variables with zero means and variances σ_{CL}^2 and σ_V^2 for CL and V, respectively. Thus σ_{CL} and σ_V represent the variability in CL and V within the population, and σ_ε represents the residual variability within each animal. The aim was to investigate how accurately and precisely the values of CL, V, σ_{CL}, σ_V, and, where possible, σ_ε could be estimated. (It was not possible to estimate σ_ε under the one sample per animal experimental conditions—insufficient information present in the data. Thus, σ_ε could only be estimated from multiple sampling designs in which more than one sample per animal was available.)

Simulation

Individual CL values (CL_js) were randomly generated by sampling from the population distribution (CL, σ_{CL}^2). V_js were similarly generated. Using the appropriate sampling time (t) sampled from the uniform distribution ($t \pm 7.5$ min), apart from the first two points, the expected concentration C_j^* was computed. A proportional error was then added to C_j^* to give the final observation. This was repeated for each animal comprising a data set.

In this simulation study, the parameter values used were $CL =$ 1.3 ml/min, $V = 162.5$ ml, and σ_{CL} and σ_V were set to give coefficients of 15 and 30%. σ_ε was set to 15% (apart from in the Varying the Error in Concentration Measurements section below).

Sampling Design

The simulated mean $t_{1/2}$ of the drug (using CL and V) was 84 min. There were 10 sampling times (i.e., 5, 15, 30, 60, 90, 120, 150, 180, 210, and 240 min). The first 2 time points were fixed, but the other points were sampled uniformly from a range of 15 min about the stated times. This was considered to mimic a real study, and in parameter estimation with NONMEM (Version II) the exact times were used.

Varying the Number of Animals Per Time Point

Let the total number of animals used in each experiment be denoted by N_A, and the total number of observations, N_S. Each of the N_A animals supplied one observation, and a different number of animals was used at each time point for different experiments. This is denoted as the $N_A * 1$ design. In the first set of experiments the effect of increasing the number of animals per time point on the accuracy and precision with which parameters were estimated was investigated. There were nine sample sizes (20, 30, 40, 50, 60, 70, 80, 100, and 150) which involved the use of 2, 3, 4, 5, 6, 7, 8, 10, and 15 animals, respectively, at each time point, and this yielded nine $N_A * 1$ study designs. The $N_A * 1$ designs were studied at two levels of variability

(15 and 30%) in CL and V in these sets of experiments with σ_ε fixed at 15%.

Varying the Error in Concentration Measurements

The influence of specified residual intraanimal variability (or error in concentration measurement) on parameter estimation was studied for three cases: $\sigma_\varepsilon = 0$, 15, and 30% with three $N_A * 1$ designs of N_S and $N_A = 30$, 50, and 70. Interanimal variability was set to 30% (i.e., σ_{CL}, $\sigma_V = 30\%$).

Repeated Measurements Design: Doubling the Total Number of Samples Per Animal with or Without Halving the Total Number of Animals

Initially, the effect of keeping N_S constant while halving N_A on parameter estimation was investigated by sampling each animal twice. The sampling regimen for this series of simulations involved dividing the 10 sampling times into two independent blocks: the first 5 times (t_1 to t_5), and the later 5 times (t_6 to t_{10}). Thus, each animal was sampled at, for example, the first times in each block (i.e., t_1 and t_6) or the second times in each block, etc. The study in which each animal was sampled twice is denoted $N_A * 2$. 15, 25, and 35 animals were used yielding three $N_A * 2$ designs with corresponding N_S of 30, 50, and 70, respectively. This allowed comparison with the $N_A * 1$ designs.

Later, the effect of keeping N_A constant while doubling N_S was investigated using $N_A = 30$, 50, and 70 animals. Each animal supplied two observations with resultant corresponding total number of observations of 60, 100, and 140, respectively. Sampling was as described in the previous paragraph.

Data Structure

For each study design, 30 sets of data were generated and analyzed assuming zero covariance between parameters.

Analysis

Prediction Error

Since "true" parameter values were known in the simulations, the accuracy and precision of parameter estimation could be quantified. Both the degree of bias and the precision of estimates relative to true values were of interest and were computed.

To express bias and precision on the same scale, percentage prediction errors were computed. For each run and for each parameter, the difference between the true value (θ_i^*) and the estimated value (θ_i) was expressed as

a percentage of the true value (i.e., percentage prediction error, $\%PE$). Thus,

$$\%PE = [(\theta_i - \theta_i^*)/\theta_i^*] \times 100 \quad (5)$$

The mean of $\%PE$ for each of 30 replicates of data was used as a measure of accuracy and SD of $\%PE$, a measure of precision of parameter estimation.

Some data sets gave rise to totally implausible estimates. Since these would be rejected from the analysis, criteria had to be adopted with which to judge acceptability. Thus any parameter estimate that was smaller than 1/100th of the true value or larger than 10 times the true value was rejected. Also, if the estimated standard error of a parameter was greater than 10 times the true value, the result was rejected. This is similar to the criteria used by White *et al.* (7) in a simulation study with a drug exhibiting one-compartment open-model kinetics.

Number of High Pairwise Correlations

The number of "high" correlation between parameter estimates was used to examine the reliability of parameter estimates. Two parameters were judged to be highly correlated if the pairwise correlation coefficient was ≥ 0.75; otherwise, it was termed low (8,9). Parameter estimates are reliable if the number of high pairwise correlation is low and the relative standard errors are low.

RESULTS

Effect of Increasing the Number of Animals Per Time Point

With the outlier criteria outlined above, 29, 29, 29, 30, 30, 30, 30, 30, and 30 acceptable NONMEM runs were obtained for the 2, 3, 4, 5, 6, 7, 8, 10, and 15 animals per time point designs, respectively.

Bias and Precision

Figure 1(a–d) summarizes the results when σ_{CL} and σ_V were set at 15%. As the number of animals per time point increased, the precision of the estimates increased as indicated by the reduction of the error bars. However, most of the estimates of CL and V were negatively biased, irrespective of the number of animals used. It was also of some interest to consider the magnitude of the SD of $\%PE$ for the various parameters. $N_A * 1$ designs yielded relatively precise estimates for the fixed effect (structural model) parameters (i.e., CL and V). If one considers a parameter to be acceptably precise when the SD of $\%PE \leq 25\%$, then estimates of σ_{CL} were acceptably precise when the number of animals at each time point was 5 or larger, but the estimates of σ_V were acceptably precise only when the number of animals

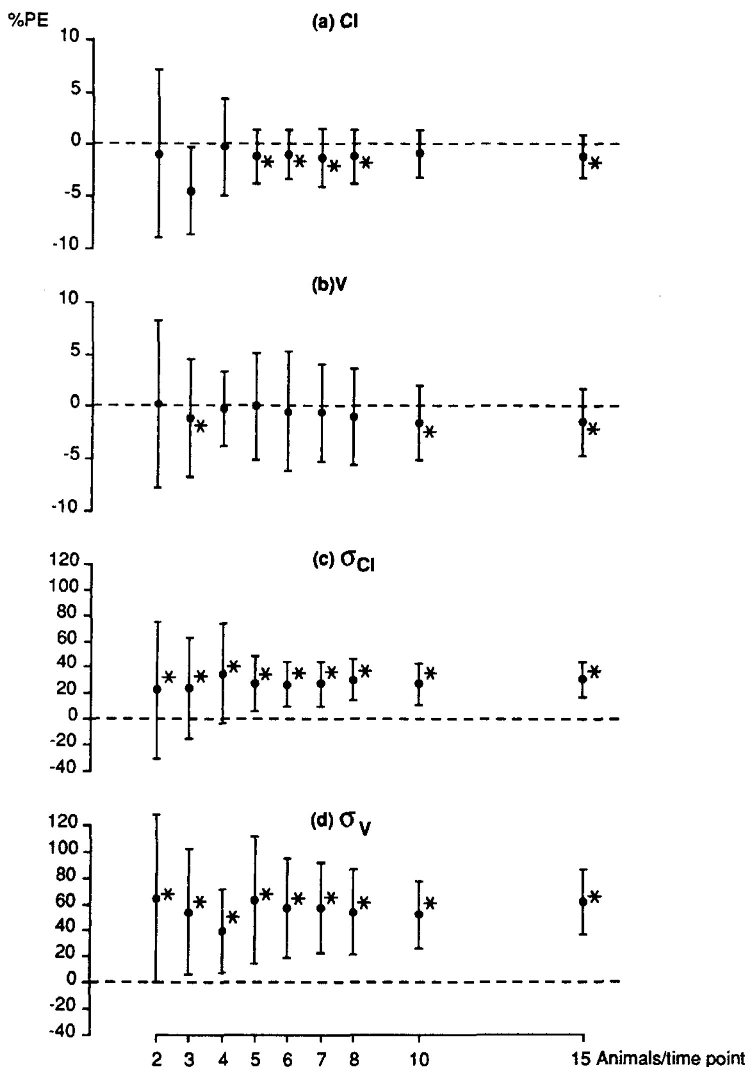

Fig. 1. Bias and precision expressed as $\%PE$ ($\bar{x} \pm$ SD, respectively) for parameters. The horizontal axis represents the number of animals used at each time point. Each vertical line expresses the bias and precision of the population parameter estimate. Only one observation was made on each animal. The interanimal variability was set at 15%, and the error in concentration measurements was set at 15%. Significant ($p<0.05$) biases are indicated by asterisks.

at each time was 10 or larger. The estimates of interanimal variability were, however, consistently positively biased and were relatively unaffected by increasing the number animals.

When σ_{CL} and σ_V were set at 30%, the estimates of the structural model parameters were negatively biased, but precise (Fig. 2, a and b). As with the 15% interanimal variability experiment, all estimates of σ_{CL} and σ_V were mostly imprecise and positively biased. Estimates of σ_{CL} with acceptable precision were obtained when the number of animals used at each time point was 7 or larger while σ_V estimates were acceptably precise when 10 animals or more were used at each time (Fig. 2, c and d). As expected, the precision with which parameters were estimated increased as the number of animals per time point increased.

Number of High Pairwise Correlations

There were no notable high pairwise correlations irrespective of the level of interanimal variability in *CL* and *V*.

Effect of Varying the Error in Concentration Measurements

There were 28, 29, 27, 30, 30, 28, 29, and 28 acceptable NONMEM runs for the following $N_A * 1$ and $\sigma_\varepsilon(\%)$ combinations: 3 and 0%, 3 and 15%, 3 and 30%, 5 and 0%, 5 and 15%, 5 and 30%, 7 and 0%, 7 and 15%, 7 and 30%, respectively. The accuracy and precision of the fixed-effect parameters were relatively unaffected by varying the error in concentration measurements. When σ_ε was 15%, the estimates of interanimal variability were less precise, as expected, and biased, and this trend was maintained for σ_ε of 30%. Moreover, the estimates were significantly positively biased (Fig. 3, a–d). The bias in the estimation of interanimal variability was unaffected by N_A (N_S).

Effect of Varying the Total Number of Samples

Bias and Precision

When N_S was kept constant while N_A was halved so that each animal supplied two concentration–time points (i.e., $N_A * 2$ designs), N_A equaled 15, 25, and 35, preserving the total number of data points (N_S). The number of acceptable NONMEM runs were 14, 18, and 16 for N_A of 15, 25, and 35, respectively. Most of the excluded NONMEM runs had spurious estimates of σ_ε. The results of the $N_A * 2$ designs are shown in Fig. 4 (a–f, second panel) with the $N_A * 1$ designs (Fig. 4a–f, first panel) included for reference. The estimation of the structural model parameters was relatively unaffected (Fig. 4, a–c). The bias in the estimation of σ_{CL} and σ_V was

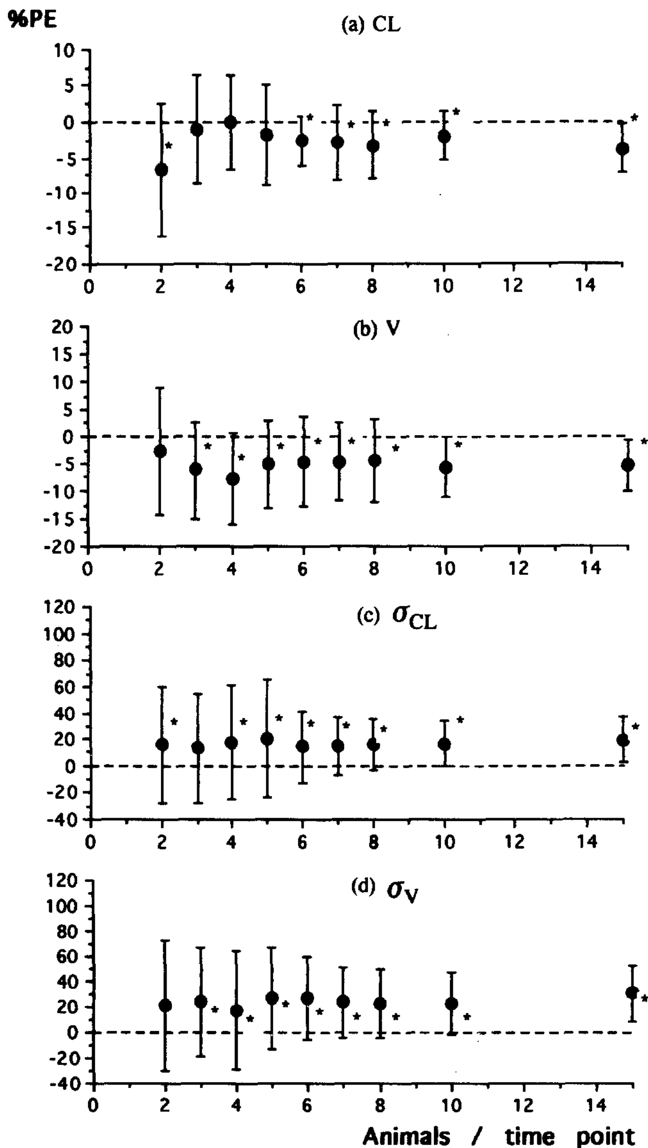

Fig. 2. Bias and precision expressed as %PE ($\bar{x} \pm$ SD, respectively) for parameters. The horizontal axis represents the number of animals used at each time point. Each vertical line expresses the bias and precision of the population parameter estimate. Only one observation was made on each animal. The interanimal variability was set at 30%, and the error in concentration measurements was set at 15%. Significant ($p < 0.05$) biases are indicated by asterisks.

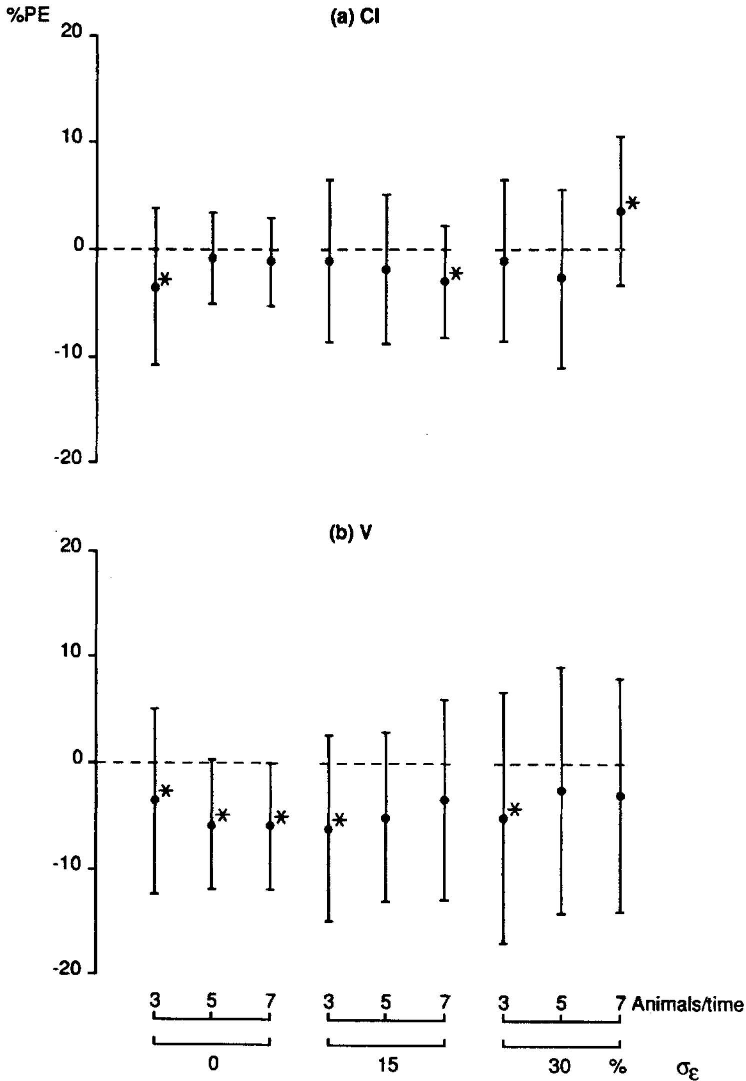

Fig. 3. Bias and precision expressed as $\%PE$ ($\bar{x} \pm$ SD, respectively) for parameters. The horizontal panels show data obtained using $\sigma_\varepsilon = 0$, 15, and 30%. Only one observation was made on each animal. Each vertical line expresses the bias and precision of the population parameter estimate. The interanimal variability used was at 30% (see Methods). Significant ($p < 0.05$) biases are indicated by asterisks.

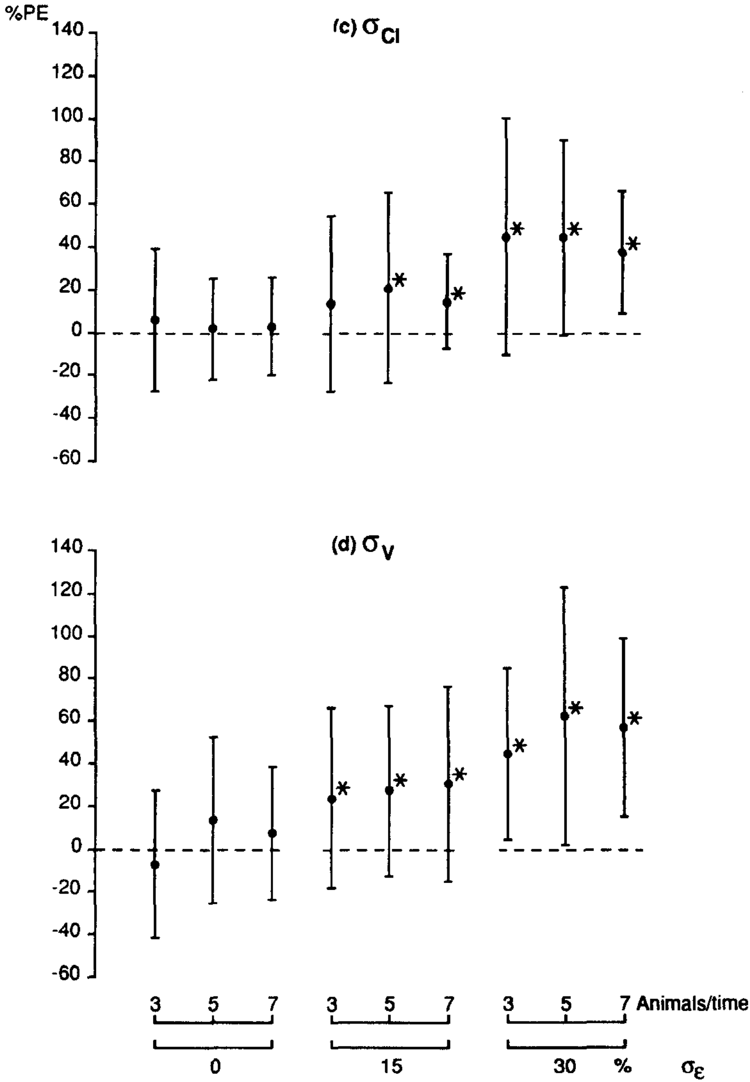

Fig. 3. Continued.

significantly reduced (Fig. 4, d–f), but the precision of the estimates was unaffected. The relatively poor precision for σ_V obtained with N_A of 35 ($N_S = 70$) as compared to 25 ($N_S = 50$) was due to some estimates being at the ceiling of the cutoff point for outliers. The bias in the estimation of σ_ε ranged from −2.9% ($N_A = 35$) to −13.7% ($N_A = 15$), and the SD of $\%PE$ from 19.5% ($N_A = 15$) to 35.9% ($N_A = 35$).

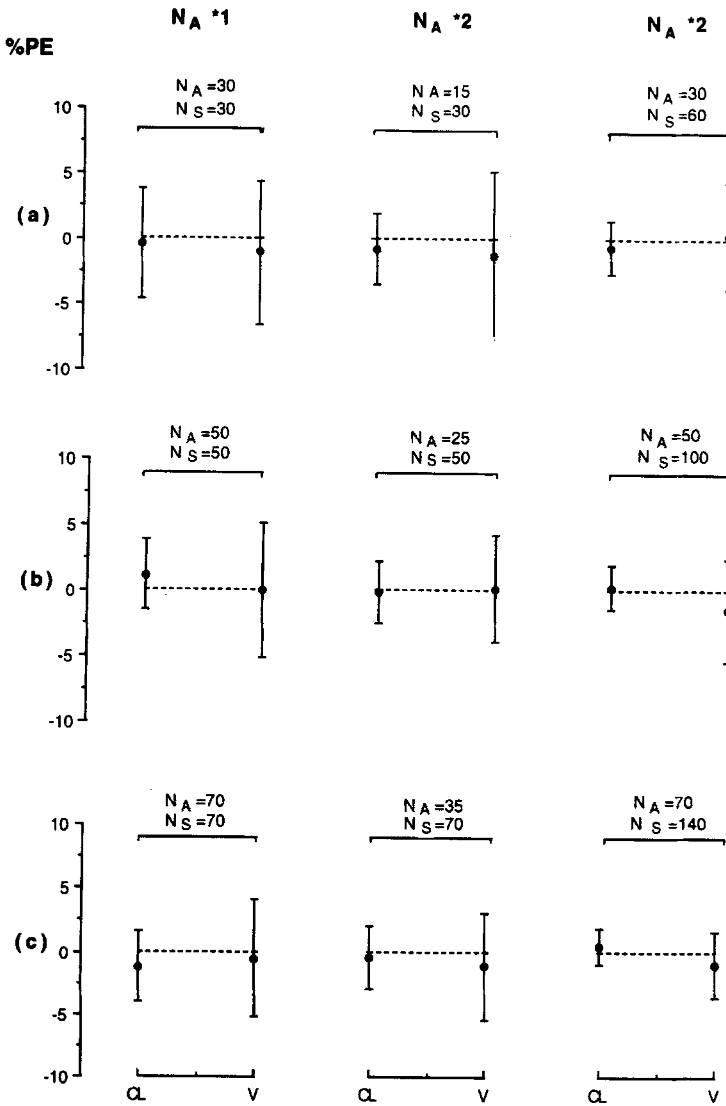

Fig. 4. Bias and precision expressed as $\%PE$ ($\bar{x} \pm$ SD, respectively) for parameters. The horizontal panels in each figure show results from different study designs. The first panel for each figure shows results with $N_A * 1$ designs which is used as a reference for comparing results obtained with $N_A * 2$ designs (second and third panels, see Methods). N_A represents the total number of animals used for each study design and N_S, the number of observations for each design. σ_{CL}, σ_V, and σ_ε were set at 15%. Significant ($p<0.05$) biases are indicated by asterisks.

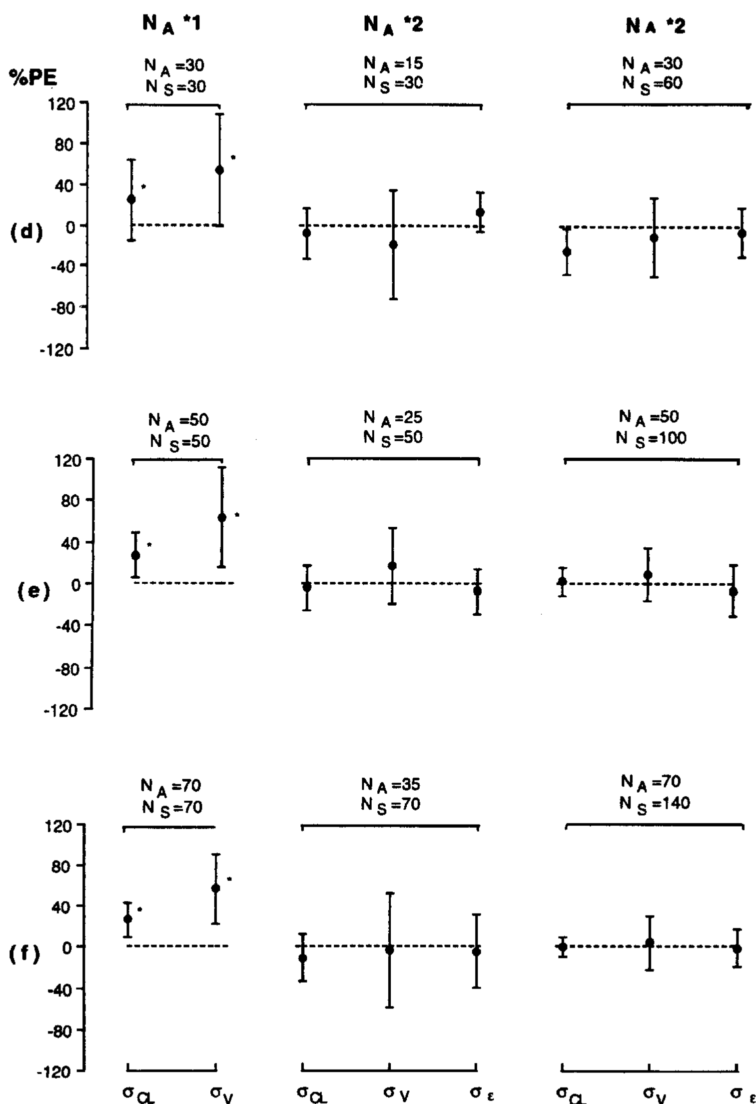

Fig. 4. Continued.

Again, with each animal supplying two concentration–time points, and fixing N_A at 30, 50, and 70 to maintain the number of animals constant allowing comparison with the $N_A * 1$ designs, acceptable NONMEM runs for this aspect of the study were 24, 23, and 28 for N_A of 30, 50, and 70, respectively. As in the previous experiment, the accuracy with which the

Table I. Number of High Correlation Values Associated with Parameter Estimates at 15% Variability in *CL* and *V* Using $N_A * 2$ Designs[a]

Parameter	No. of high pairwise correlations (%)					
	$N_A = 15$	$N_A = 25$	$N_A = 30$	$N_A = 35$	$N_A = 50$	$N_A = 70$
V vs. CL	0.0	0.0	0.0	0.0	0.0	0.0
σ_{CL} vs. CL	13.3	0.0	0.0	0.0	0.0	0.0
σ_{CL} vs. V	6.7	0.0	0.0	0.0	0.0	0.0
σ_V vs. CL	0.0	0.0	0.0	0.0	0.0	0.0
σ_V vs. V	6.7	0.0	6.3	5.2	0.0	0.0
σ_V vs. σ_{CL}	33.3	21.1	0.0	3.8	4.4	3.6
σ_ε vs. CL	100.0	100.0	100.0	100.0	100.0	100.0
σ_ε vs. V	6.7	0.0	0.0	0.0	0.0	0.0
σ_ε vs. σ_{CL}	0.0	0.0	0.0	0.0	0.0	0.0
σ_ε vs. σ_V	40.0	47.4	6.3	9.1	13.0	14.3

[a]Correlation coefficient ≥ 0.75. N_A = Number of animals (sampled twice).

fixed-effect parameters were estimated was relatively unaffected, but precision was improved as expected (Fig. 4, *a–c*, third panel). The bias in the estimates of σ_{CL} and σ_V was almost completely eliminated and precision greatly improved (Fig. 4, d–f, third panel). However, acceptable estimates of σ_{CL} and σ_V were only obtained with N_A of 50 (SD of $\%PE = 17.7\%$) and 70 (SD of $\%PE = 24.6\%$) (i.e., $N_S = 100$ and 140, respectively). Again, spurious estimates of σ_ε were responsible for the exclusion of most NONMEM runs.

Number (%) of High Pairwise Correlations

When N_A was halved for the $N_A * 2$ design, the largest number (i.e., 100%) of high pairwise correlation was observed between σ_ε and CL irrespective of N_A (Table I). In addition, the percentages of high correlation between σ_{CL} and σ_V obtained for N_A of 15, 25, and 35, were 33.3, 21.1, and 3.8%, respectively, while 40.0, 47.4, and 9.1% was obtained with N_A equal to 15, 25, and 35, respectively, for the correlation between σ_ε and σ_V. The slightly higher number of high pairwise correlation obtained for N_A of 25 compared to N_A of 15 was due to some NONMEM runs for N_A of 25 having parameter estimates at the ceiling of cutoff points for acceptability of estimates. Parameter estimates were more highly correlated with each other when N_A was 15 than 25 or 35.

Without halving N_A for $N_A * 2$ design, σ_ε and CL were highly correlated irrespective of N_A (Table I). Except for the correlation between σ_ε and σ_V in which the percentage of high correlation ranged from 6.3 ($N_A = 30$) to 14.3 ($N_A = 70$), and the correlation between σ_V and V where the value was 6.3% for N_A equal to 30, the number of pairwise correlations was less than 5%.

DISCUSSION

We have carried out a simulation study to examine the effects of some design features on the estimation of population pharmacokinetic parameters in a preclinical animal setting. Sampling strategies that are applicable to small laboratory animals such as mice were studied in addition to the influence of assay error. Under the conditions studied, the structural model parameters were well estimated irrespective of the interanimal variability studied for most $N_A * 1$ designs. The accuracy of these estimates was relatively unaffected by increasing the number of animals per time point. The positively biased estimates of interanimal variability highlights the difficulty in estimating this aspect of variability when there is no information about one of the components of variability (in this case, σ_ε). This emphasizes the limitation of the one point per animal design. Estimates of variability associated with structural model parameters are considerably less precise, given a fixed number of experimental animals, than are estimates of their means. Some significant biases, associated with parameter estimates obtained with designs having a larger number of animals compared to the ones with fewer animals at each time point, were due to samples being large enough to detect bias.

Estimates of σ_{CL} and especially σ_V were much larger when their true values were set at 15% than at the 30% level because when the true standard deviation of the parameter is decreased, the deviation of the estimated values from it is lowered more slowly.

Since estimates were considered acceptably precise when the SD of $\%PE \leq 25\%$, the minimum number of animals required for reasonable estimation of population pharmacokinetic parameters with the one observation per animal design was 100 (10 animals/time) with the settings of interanimal variability considered.

When the interanimal variability was between 15 and 30%, *CL* and *V* were accurately and precisely estimated with 4 to 15 animals used per time point. Thus, as few as 4 animals per time could be used for the estimation of the structural model parameters with these settings of interanimal variability. σ_{CL} and σ_V were poorly estimated with all $N_A * 1$ designs due to a lack of information about σ_ε. It stands to reason, therefore, that the $N_A * 1$ design is good enough for the estimation of structural model parameters, and this is where it finds its application.

When σ_ε was varied to examine its effect on the estimation of σ_{CL} and σ_V, the magnitude of bias in these parameters increased with the magnitude of σ_ε, indicating that a substantial fraction of this bias was due to an error, i.e., the residual intraanimal error, which could not be partitioned. This finding confirms an earlier observation by Graves *et al.* (10). Using Monte

Carlo simulation techniques, these authors generated data sets with error in concentration measurements without introducing intersubject variability, and concluded that error in concentration measurements contributes significantly to large standard deviations associated with structural model parameters which could be interpreted as interindividual variability in a real study situation. In fact, the estimates of interanimal variability produced by NONMEM with the $N_A * 1$ designs were composites of inter- and residual intraanimal variability.

Most NONMEM structural model parameter estimates derived from all studies with the $N_A * 1$ designs showed a consistent significant negative bias, generally less than 10%. This was due to estimation error (a consequence of the first-order approximation) as negative biases in the estimation of these parameters were obtained even when σ_ε was set at 0%.

A tradeoff between sample size and the total number of animals (i.e., doubling the total number of observations (sampling an animal twice) while reducing the total number of animals sampled by half) produced a dramatic improvement in the estimation of interanimal variability with a considerable reduction in bias. Accuracy was stable over the different population samples. The second sample practically eliminated bias and facilitated the partitioning of interanimal variability and residual error, by introducing information about σ_ε. However, the estimates of σ_ε were unstable probably because of the correlation of σ_ε with CL and σ_V, while the estimates of σ_{CL} and σ_V were relatively stable. There were more high pairwise correlations between σ_ε and σ_V for N_A of 15 and 25 than 35 (Table I).

Keeping N_A constant as in the $N_A * 1$ designs while doubling N_S ($N_A * 2$ designs) resulted in a significant improvement in the precision with which interanimal variability was estimated. This had no effect on accuracy and precision of the structural model parameters. The estimates of σ_ε were more stable with significant high correlations occurring only between σ_ε and CL.

Doubling the number of observations per animal results in savings in terms of the number of animals that are needed in this type of study. The $N_A * 2$ design with N_A of 50 animals yielded acceptably precise estimates of interanimal variability without compromising accuracy. The use of this minimal number of animals with the $N_A * 2$ design and sampling strategy considered here would result in savings not only in animal number but also in time and labor cost without sacrificing the accuracy and precision with which population pharmacokinetic parameters are estimated.

Given the design specifications considered here, accuracy and precision in the estimation of interanimal variability is significantly improved when the data set is enhanced by taking two observations per animal. Experimental methods have become available which permit serial blood sampling in small laboratory animals (11). These sampling methods combined with modern

approaches to population data analysis should lead to more informative pharmacokinetic studies in small animals.

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