Accuracy and Precision of Pharmacodynamic Exponent

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INTRODUCTION

Identification of optimal dosing regimens is an important field of study in cancer chemotherapy. In the empirical pharmacodynamic relationship between the drug concentration (C) and exposure time (T) and the drug exposure constant (h), $C^n \times T = h$, h is a constant related to the drug effect. The pharmacodynamic exponent n indicates the relative importance of C and T in determining the pharmacologic effect (1–4). We have shown that analysis of the C vs T plot for a given effect (e.g. 50% of inhibition of cell proliferation) provides the n value (3,4). When the n value equals 1.0, the relationship collapses to $C \times T = h$, and C and T are inversely related and contribute equally to the effect. In this case, treatment schedules that produce the same $C \times T$, regardless of the shape of the concentration-time profile (e.g. short exposure to high concentrations or long exposure to low concentrations), will result in identical effects. When the n value is greater than 1.0, the C^n term contributes more than T to the effect. In this case, a short infusion that delivers high concentrations will produce a greater effect than a long infusion that delivers the same $C \times T$ but lower concentrations. The reverse is true when n is less than 1.0.

When the n value deviates from 1.0, even at a relatively minor extent, different treatment schedules can result in large differences in the $C^n \times T$ product. For example, two hypothetical concentration-time profiles, i.e. 10 μg/ml for 1 hr or 1 μg/ml for 10 hr, would yield identical $C^n \times T$ of 10 μg^n-hr/ml^n at an n value of 1.0 but significantly different $C^n \times T$ values of 17.0 and 10.0 μg^n-hr/ml^n, respectively, at an n value of 1.23. Hence, the precision and accuracy of the n value obtained from pharmacodynamic analysis as well as the statistical significance of the n value (i.e. whether the estimated n is significantly different from 1.0) are important for deciding on the treatment schedules that produce the highest effect.

We have shown that the accuracy, precision, and statistical significance of the n value depend on the experimental design, method of data analysis, and tumor sensitivity to the drug (4). However, the relationship between the variability in the n value and the variability in the effect data is not known. As an example, if the effect data show a standard deviation (SD) of 20%, is the n value of 1.20 significantly different from 1.0? In a typical pharmacodynamic study, the drug effects at multiple treatment times are determined using several replicates at each time point. These data provide a measurement of the variability in the effect data (e.g. SD of multiple observations), but not the variability in the n value because n is a calculated parameter derived from analysis of the concentration-effect relationship.

The goal of the present study was to determine the effects of two factors, i.e. the numerical values of the n estimates and the magnitude of variability in the effect data, on the accuracy, precision, and statistical significance of the n estimates. This study used the data generated by relatively few experiments together with Monte Carlo simulations to generate additional data sets with comparable variability as in the experimentally obtained data. The Monte Carlo simulations, based on the variability in the effect data, provided the variability in the n value and thereby the evaluation of the statistical significance of the experimentally determined n value. The use of the Monte Carlo simulations circumvents the need of defining the mathematical relationship between the variabilities in the effect data and the n values.

MATERIALS AND METHODS

Experimental Plan

The step-wise research plan was as follows: (a) select the pharmacodynamic model and the model parameters, including the n values (referred to as specified n values), (b) use Monte Carlo simulations to generate 100 sets of drug concentration-treatment time-effect data for each of the different combinations of model parameters, (c) analyze the simulated data to obtain n estimates (referred to as estimated n values), and (d) compare the specified n values with the average of the 100 estimated n values obtained for each condition to determine the accuracy, precision and statistical significance of the estimated n values.

Pharmacodynamic Model

Drug effect was determined by the sulforhodamine B (SRB) assay, which measures the cellular protein level and is a surrogate measurement of the cell number. Analysis of pharmacodynamic data was performed using the 3-dimensional surface response as described by Equation 1(4).

$$E = E_0\left(1 - \frac{C^m}{(h/T)^{m/n} + C^m}\right) \quad \text{where} \quad \left(\frac{h}{T}\right)^{1/n} = IC_{50} \tag{1}$$

E_0 is the baseline value in the absence of drug and is the SRB reading for the untreated controls. E is the SRB reading for drug-treated samples. E_0 and E are expressed as % of the control value. Because E_0 is a fitted parameter, its value can exceed 100%. n is the pharmacodynamic exponent in the empirical

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ABBREVIATIONS: C, drug concentration; CV, coefficient of variation; E, drug effect; h, exposure constant; IC_{50}, drug concentration that produces 50% effect; MMC, mitomycin C; m, slope parameter of concentration-effect relationship; n, pharmacodynamic exponent; SD, standard deviation; SRB, sulforhodamine B; T, exposure time.

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relationship of $C^n \times T = h$. h is the drug exposure constant. IC_{50} is the drug concentration that produces 50% effect. h is proportional to $(IC_{50})^n$ when T remains constant, and a high h value indicates high IC_{50} and therefore a lower tumor sensitivity (4). m describes the shape of the surface response and a lower value of m is associated with a more shallow concentration-effect relationship. Equation 1 was derived by combining the empirical equation $C^n \times T = h$ and the sigmoid E_{max} model that describes a concentration response curve (4). The assumption for the model described in Equation 1 is that a given drug exposure will produce a pharmacologic effect of up to 100% (as opposed to an incomplete effect of <100%). We have shown that analysis of data by this surface response relationship provides more accurate and precise estimates of n, compared to a two-step method in which the IC_{50}'s are first calculated for each exposure time and then fitted with the $C^n \times T = h$ equation to solve for the n value (4).

Pharmacodynamic Model Parameters

For the specified n values, we arbitrarily selected 21 n values distributed between 0.6 to 1.4. The other parameters described in Equation 1, i.e. E_0, h, and m were identical to those obtained from the experimental data on the pharmacodynamics of mitomycin C (MMC) in human pharynx FaDu cancer cells (i.e. E_0 = 100%, h = 6.55, and m = 1.02; see Figure 1). The model parameters were obtained from 4 experiments of 5 concentration-response curves determined for 5 treatment durations. Each concentration-response curve consisted of 9 data points, with 6 replicates per point. A total of 63 permutations containing different combinations of n values (21 values) and effect data variability (3 levels), were studied.

Monte Carlo Simulations

The previously described Monte Carlo method (4) was used to generate the concentration-response data using Equation 1. A two-step procedure was used to generate effect data which contain variability (E_{var}) that is representative of the variability observed experimentally. First, error-free effect data with no variability (E_{calc}) were generated using different drug concentrations (ranging from 0.001 to 100 μg/ml) and different treatment times (ranging from 1.5 to 48 hr). The simulations were performed using different specified n values. Second, a variability term, SD (standard deviation), is added to E_{calc} to generate the variability-containing E_{var} data using Equation 2.

$$E_{var} = E_{calc} + SD * Rannor(x) \quad (2)$$

Rannor(x) values are normally distributed random numbers with a mean of 0 and a standard deviation of 1. The SD was derived from the experimental data (see below).

The experimental variability in the effect measurement was dependent on the magnitude of the effect. A comparison of the relationships of the effect with several measures of variability, i.e. SD, CV, and log or reciprocal of the CV, showed the linear (inverse) relationship between effect and CV yielded the highest coefficient of determination (r^2 = 0.56) and the lowest Akaike Information Criterion value. The CV declined with increasing drug effect. When the effect was minimal (i.e., <5%), some CV values were exceedingly large. CV values larger than twice the intercept of the CV vs E plot were considered outliers and were not included in the analysis. We defined three levels of variability, i.e., low, middle, and high, where the middle level represents the linear regressed line for the plot of CV vs E, and the low and high levels of variability represent the lower and upper limits of the 95% prediction interval. The ranges of CV for the low, middle and high levels of variability were 0 to 10.5%, 0 to 21.6%, and 10.4 to 32.7%, respectively (Fig. 1A). The corresponding intercepts for the three lines were 10.6%, 21.7%, and 32.8%, respectively. The three lines showed almost identical slopes of about −0.18.

The low, middle, and high levels of the intercepts and slopes of the CV vs E plots were used to calculate three levels of SD as a function of the E value, as follows. E_{mean} is the mean E value.

$$CV = \frac{SD}{E_{mean}} \times 100\% = (slope * E + intercept) \quad (3)$$

$$SD = \frac{(slope * E + intercept) * E_{mean}}{100\%} \quad (4)$$

Equations 2 and 4 were used to simulate E_{var}. For each combination of different pharmacodynamic model parameters, 100 concentration-response data sets with six replicates were generated. The simulated data were then analyzed using Equation 1 to obtain the estimated n values.

Table 1. Comparison of Variability in n Values Obtained from Experimental and Simulated Data

Values of n	Experimental data	Simulated data	
		Middle level	High level
No. of experiment	4	100	100
Mean	1.11	1.11	1.11
Median	1.13	1.11	1.11
SD	0.06	0.03	0.06
CV	5.07	2.42	5.04
Range	1.04–1.16	1.05–1.19	0.99–1.29

Note: FaDu cells were treated with MMC in 4 experiments, using 6 replicates per data point. The CV in the effect data was determined from the 6 replicates. For the middle and high levels of variability, the middle and the upper lines in Figure 1A were used to simulate data sets. Differences between the mean n values obtained from experimental and simulated results were not significant (two-tailed Student's t-test).

Determination of Accuracy, Precision and Statistical Significance of Estimated n Values

The difference between the specified n value and the average estimated n value indicated the accuracy (i.e., a smaller difference indicated a greater accuracy). The CV of the estimated n values indicated the precision (i.e., a greater CV indicated a lower precision). The fraction of the 100 estimated n values falling above or below 1.0 was determined for each specified n value used for the simulations. This fraction indicates the frequency of correctly identifying n values as greater or less than 1.0, and therefore the statistical significance of the specified n value.

Simulation and Data Analysis

Simulation of data sets and nonlinear estimation of pharmacodynamic parameters were performed using SAS (SAS

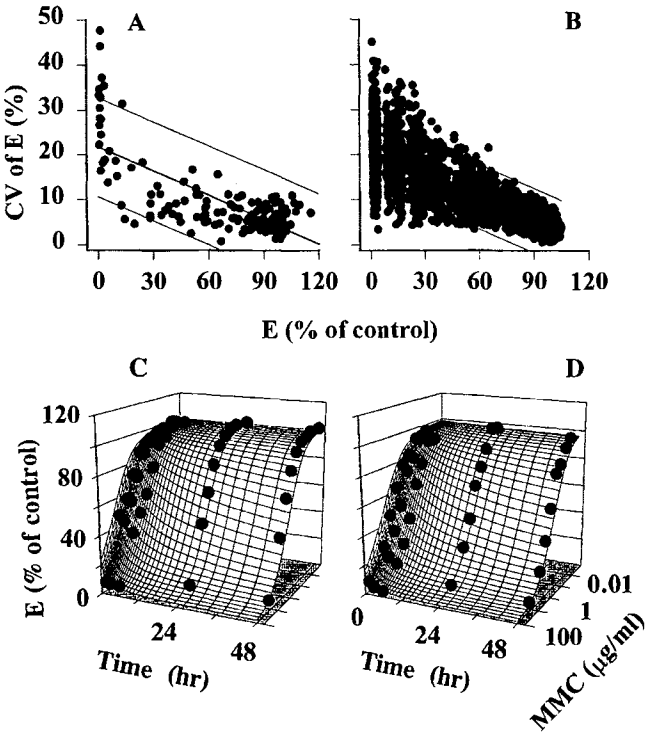

Fig. 1. Comparison of experimental and simulated data. (A) The coefficient of variation of E as a function of E is shown for the experimental data. Each data point represents a CV of E for 6 replicates for each experiment. Data for 4 experiments are shown. The middle line is the least squares linear regression line for the middle level of variability, i.e., $y = 21.7\% - 0.18\ E$ ($r^2 = 0.56$, $p < 0.0001$). The top and bottom lines are the upper and lower limits of the 95% prediction interval of the data points (intercepts of 32.8% and 10.6%, respectively). (B) Variability simulated using the middle level of intraday variability of the experimental data. The least squares linear regression line for the middle level of variability is $y = 20.9\% - 0.017\ E$ ($r^2 = 0.75$, $p < 0.0001$). The top and bottom lines are the upper and lower limits of the 95% prediction interval of the data points (intercepts of 27.9% and 13.8%, respectively). (C) Experimental data analyzed by the surface response method. Each data point represents the mean of 6 replicates of a randomly selected experiment. The mesh surface was obtained from nonlinear parameter estimation according to Equation 1. The resulting parameter estimates are: $E_0 = 102\%$, $m = 1.18$, $h = 6.52$, $n = 1.04$. (D) Simulated data of a randomly selected experiment, analyzed by the surface response method. The resulting parameter estimates are: $E_0 = 99\%$, $m = 1.01$, $h = 6.42$, $n = 1.12$.

Institute, Inc., Cary, NC) on a pentium-based personal computer. Marquardt's method was used for all nonlinear estimations of parameters. Statistical analysis of the differences between the specified n values and the estimated n values was performed using the two-tailed Student t test.

RESULTS

Simulated Versus Experimental Data

The assumption used in our pharmacodynamic analysis method was that the 100 data sets generated by the Monte Carlo simulations contained random variabilities that were comparable to the variabilities observed in the experimental data. This assumption was validated by the results shown in Fig. 1A and 1B and Table 1. The variability of the simulated data was, similar to that in the experimental data, linearly and inversely correlated with the effect level. These results also show that for the middle level of variability, the slope and intercept of the least squares regression line of the CV vs E plot for the simulated data was nearly identical to those for the experimental data.

Figure 1c shows the analysis of the experimentally determined pharmacodynamic data by the 3-dimensional surface response method, and Fig. 1D shows the analysis of the simulated pharmacodynamic data. The simulated results are comparable to the experimental data, as indicated by the similar curve shape and the similar pharmacodynamic parameters including E_0, m, h and n.

Accuracy and Precision of Estimated n Values

Figure 2 shows the accuracy and precision of the estimated n values, as a function of the numerical value of n and as a function of the variability in the effect data. For all of the 63 permutations (i.e., 21 n values and 3 levels of effect data variability), the differences between the specified n values and the average of 100 estimated n values were <1.2%. At the low, middle and high levels of data variability, the average estimated n values deviated from the specified n values by 0.03%, 0.1%, and 0.9%, respectively. Hence, the accuracy was >99%. The precision decreased with increasing level of variability in the simulated effect data; the average CV were 0.3, 2.4, and 5.0% for the low, middle, and high levels of variability, respectively. An interesting observation is that the precision decreased with increasing n value. For example, at the high level of variability, the CV at an n value of 1.4 was nearly twice the CV at an n value of 0.6. Because the n value reflects the convexity of the relationship between IC_{50} and the treatment duration, the lower precision at the higher n values is probably due to the greater variability in data fitting associated with a greater convexity.

Statistical Significance of Estimated n Values

Figure 3 shows the plot of (frequency of obtaining an estimated n value of either greater or less than 1.0 from the

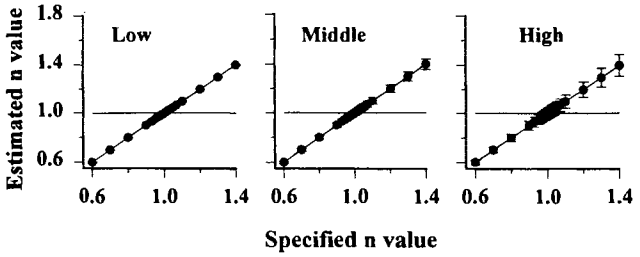

Fig. 2. Accuracy and precision of estimated n values as a function of numerical values of n and as a function of variability in effect data. Data were simulated using 21 specified n values ranging from 0.6 to 1.4, $h = 6.55$, $m = 1.02$ and the three different levels of intraday variability corresponding to the three lines in Figure 2A and calculated by Equation 5. The simulated data were analyzed by Equation 1 to yield the estimated n values. Each data point represents the mean of 100 n values estimated from 100 simulated data sets. Mean ± SD. The lines connect the data points and are not regressed lines. Regression analysis showed positive correlations at all three levels of variability ($r^2 > 0.999$, $p < 0.0001$).

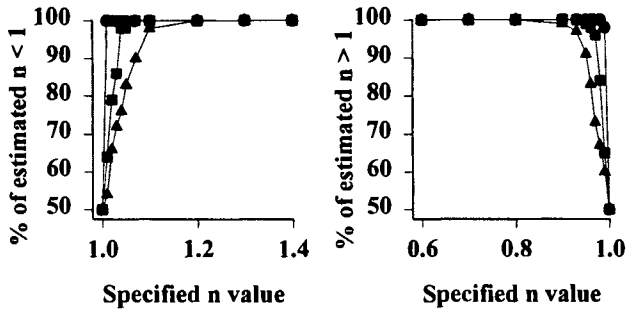

Fig. 3. Frequency of correctly identifying n estimates that deviate from 1.0. The specified n values were used to generate the simulated data sets from which the estimated n values were obtained. The frequency of the estimated n values to fall below or above 1.0 was determined for each specified n value, and for each of the three levels of effect data variability, i.e. low (●), middle (■), and high (▲). The lines connect the data points.

simulated data) vs (specified n values used to generate the simulated data), as a function of the specified n values and the effect data variability. The results indicate that the frequency for correctly identifying n values of greater or less than 1.0 increased with the difference between the n estimate and 1.0, and decreased with increasing variability in the effect data. For example, at the middle level of effect data variability, the frequency increased from 54% at the n value of 1.00 to 100% at the n value of 1.04. At the low, middle and high levels of variability, the specified n values needed to correctly identify estimated n values of greater than 1.0 at a >95% frequency (i.e. $p < 0.05$) were 1.01, 1.03, and 1.10, respectively.

Accuracy and Precision of Other Parameters

The parameters E_0, m, and h were estimated for each simulated data set. These parameters showed accuracy and precision of a similar magnitude as n. All accuracies were >99%. The CV for E_0 and m were <2% and <7%, and independent of the n value. The CV for h was about 50% larger than that of n, and increased in parallel with the CV of n.

DISCUSSION

The present study used Monte Carlo simulations, together with the variability in the drug effect data, to determine the variability in n which is a calculated parameter. We have shown that analysis of pharmacodynamic data can provide a quantitative measurement of the pharmacodynamic exponent n, which in turn indicates the relative importance of drug concentration and treatment time on drug effect (3,4). The present study demonstrated that the accuracy, precision and statistical significance of the n estimates depend on the numerical value of the n estimates and the variability in the effect data, and on the quantitative relationships between these parameters.

The inherent limitation of our approach is that the conclusions depend on the Monte Carlo simulations depend on the correctness of the pharmacodynamic model depicted in Equation 1. There are alternative approaches which do not require simulations but rather depend on resampling of the observed experimental data to identify the variability of the derived parameters. An example is the bootstrapping method (5). However, these methods are limited in that the resampling is performed on existing data, cannot be used to establish the variability of other systems where there are no experimental data, and are therefore inadequate for the purpose of the present study.

In conclusion, results of the present study show that within the usual data variability (i.e. CV of up to ~20% for 6 replicates), the n value can be obtained by the surface response method with >99% accuracy and >95% precision and an n value of 1.03 is significantly different from 1.0. We recommend performing multiple experiments to identify the highest intraday variability, as a conservative measurement. We also recommend using the high level of variability because our results indicate that this variability yielded simulated data that are comparable to the experimental data (Table 1), and because it gives the most conservative results on the accuracy and precision of the estimated n values.

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