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Comparative evaluation of six techniques for determining the Michaelis-Menten parameters relating phenytoin dose and steady-state serum concentrations

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Using randomly generated data within set error limits, the Michaelis-Menten parameters D_{max} and K_m relating steady-state serum phenytoin concentrations (C_{ss}) and dose (D) were determined by 5 graphical techniques and an iterative computer fit to the hyperbolic equation. The best mean estimates of D_{max} and K_m were provided by the latter. Assuming the experimental error to be in C_{ss} the results indicate that the most reliable graphical technique is that based on the equation $C_{ss} = D_{max}\frac{C_{ss}}{D} - K_m$. However, considering its obvious simplicity and relative reliability the direct linear plot is recommended for clinical use.

The elimination from the body of the antiepileptic drug phenytoin is unlike that observed with most drugs as it is describable pharmacokinetically by the Michaelis-Menten equation (Gerber & Wagner 1972; Atkinson & Shaw 1973; Mawer et al 1974). Consequently, the curvilinear relation between steady-state serum concentration and maintenance dose poses practical therapeutic problems for the clinician since a small increase in dose results in a disproportionately large increase in the steady-state serum concentration, the extent of which varies from patient to patient (Bochner et al 1972; Mawer et al 1974; Richens & Dunlop 1975). To achieve optimal antiepileptic treatment the monitoring of serum phenytoin concentrations (therapeutic range = 10–20 mg litre^{-1}) has become an accepted aspect of phenytoin therapy (Buchthal et al 1960; Kutt & McDowell 1968; Mawer et al 1974).

Recently three main pharmacokinetic techniques based on Michaelis-Menten principles have been reported as a means of facilitating the attainment of optimal individualized dosage: i) A nomogram based on a mean (19 patients from two studies) K_m value (Richens & Dunlop 1975); ii) A linear rearrangement of the Michaelis-Menten equation (Hofstee 1952; Dowd & Riggs 1965) in which K_m and V_{max} determined from two known steady-state concentrations on different doses are substituted in the Michaelis-Menten equation to calculate the dose for a desired concentration (Ludden et al 1977): and iii) A direct linear plot (DLP) technique (Eisenthal & Cornish-Bowden 1974) which also requires at least two different steady-state concentration-dose points but involves no calculations (Mullen 1977, 1978).

In the present work, the DLP and four linear transformations of the Michaelis-Menten equation are compared with each other and with a hyperbolic fit for their capacity to determine the best Michaelis-Menten parameters applicable to the relationship between steady-state serum phenytoin concentrations and dose.

Background

In enzyme kinetic work the classical Michaelis-Menten equation,

$$v = \frac{V_{max} \cdot C}{K_m + C} \quad .. \quad .. \quad (1)$$

relating reaction rate, v and substrate concentration, C is usually rearranged to a linear form in order to determine readily the parameters V_{max} (the theoretical maximal rate) and K_m (the value of C at $\frac{1}{2}$ V_{max}), The following three linear equations are widely employed in enzyme kinetics (Hofstee 1952; Dowd & Riggs 1965).

$$\frac{1}{v} = \frac{1}{C} \cdot \frac{K_m}{V_{max}} + \frac{1}{V_{max}} \quad .. \quad .. \quad (2a)$$

$$\frac{C}{v} = \frac{1}{V_{max}} \cdot C + \frac{K_m}{V_{max}} \quad .. \quad .. \quad (2b)$$

* Correspondence.

$$v = -K_m \cdot \frac{v}{C} + V_{max} \quad .. \quad .. \quad (2c)$$

Several papers (Hofstee 1952; Dowd & Riggs, 1965; Cornish-Bowden & Eisenthal 1974) have dealt with the relative merits of the various linear forms of the Michaelis-Menten equation. Foremost amongst these is the report by Dowd & Riggs (1965) in which it was demonstrated that the Lineweaver-Burk (or double reciprocal) plot (eqn (2a)) is far inferior to the other two transformations. Moreover, using computer simulated data containing randomly generated errors, these workers found that the plot of v (ordinate) against v/C (abscissa) (equation (2c)) produced estimates of K_m and V_{max} which were more reliable than those obtained by the other two equations. It was pointed out that the different capacity of the three equations to provide reliable estimates of K_m and V_{max} was due to the fact that the experimental error(s) normally inherent in the measurement of v would be 'magnified' in equations (2a) and (2b) by the presence of v in its reciprocal form (Dowd & Riggs 1965).

In the relation between dose and concentration of phenytoin (Fig. 1) however, the steady-state serum concentration C_{ss} (mg litre^{-1}) is the dependent (and

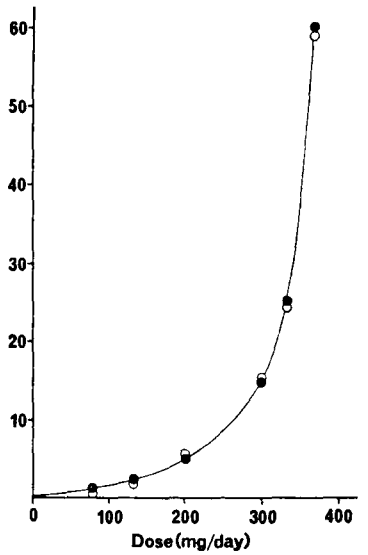

FIG. 1. The relation between steady-state concentration, Css and dose, D similar to that seen in phenytoin therapy. The 'true' concentrations (●) on the arbitrarily chosen doses indicated were calculated using equation (3) with K_m and D_{max} values of 5·0 mg litre^{-1} and 400 mg day^{-1}, respectively. Also shown on the same doses are typical ('experiment' 25) concentrations (○) generated by computer within set error limits. Ordinate: serum phenytoin concentration (mg litre^{-1}).

error containing) variable and hence equation (1) may be rearranged in the form (Mullen 1977; 1978),

$$C_{ss} = \frac{D \cdot K_m}{D_{max} - D} \quad .. \quad .. \quad (3)$$

where, v has been replaced by D (dose in mg day^{-1}) so that V_{max} becomes D_{max} (the theoretical maximal daily dose) — K_m being the steady-state serum phenytoin concentration existing at half D_{max}. (It is important to bear in mind that equation (3) only holds when $D_{max} > D$ since the steady-state would not be achieved in the event of D being greater than D_{max}. Furthermore, in the actual clinical situation, true determinations of K_m and D_{max} are obtained only when average steady-state concentration, $\bar{C}_{ss}$ values are used (Wagner 1978)).

Analogous to equations (2a), (2b) and (2c) three linear forms of equation (3) can be written, each having C_{ss} (or its reciprocal) as the dependent variable:

$$\frac{1}{C_{ss}} = \frac{D_{max}}{K_m} \cdot \frac{1}{D} - \frac{1}{K_m} \quad .. \quad .. \quad (4a)$$

$$\frac{D}{C_{ss}} = -\frac{1}{K_m} \cdot D + \frac{D_{max}}{K_m} \quad .. \quad .. \quad (4b)$$

$$C_{ss} = D_{max} \cdot \frac{C_{ss}}{D} - K_m \quad .. \quad .. \quad (4c)$$

Since the experimental (analytical) error is inherent in C_{ss}, intuitive reasoning suggests on the basis of the findings of Dowd & Riggs (1965) that equation (4c) above should provide the most reliable estimates of K_m and D_{max}.

METHODS

Computer simulated data were used to test this hypothesis. The values of D_{max} and K_m obtained from equations (4a), (4b) and (4c) were compared with each other and with those obtained by the DLP technique (Mullen 1977; 1978) and by an iterative computer fit based on the hyperbolic equation (3). In addition the same data were utilized to determine D_{max} and K_m by the method of Ludden et al (1977) using equation (2c) in which D and D_{max} were substituted for v and V_{max}, respectively.

In this investigation it is assumed that each dose, D, is so controlled that no error exists in this variable. (In the clinical setting this may not always be the case due to non-compliance by the patient and changes in the effective dose resulting from altered drug absorption, etc.). In addition, the observed

steady-state serum phenytoin concentrations C_{ss}, produced by each dose are considered to be normally distributed about a 'true' value of C_{ss} calculated using equation (3). For this purpose K_m and D_{max} were arbitrarily assigned 'true' values of 5·0 mg litre^{-1} and 400 mg day^{-1}, respectively—both values being representative of those reported in patients. The following six 'true' concentrations (mg litre^{-1}) were utilized (the corresponding calculated doses (mg day^{-1}) are in parentheses): 1·25 (80·0), 2·5 (133·3), 5·0 (200·0), 15·0 (300·0), 25·0 (333·3) and 60·0 (369·2).

In each of the thirty 'experiments' carried out, computer generated concentrations within set error limits about the true C_{ss} values were used. For each of the six doses the error in the corresponding concentrations was selected randomly from a normal distribution centered on zero based on actual standard errors obtained from repeatedly measured (g.l.c.) steady-state concentrations in patients as found by Richens & Dunlop (1975). The percentage coefficient of variation ranged from 56·4% for the lowest (i.e. 1·25 ± 0·71 mg litre^{-1}) to 6·6% for the highest (i.e. 60 ± 4·0 mg litre^{-1}) 'true' concentration value.

Unweighted data from each 'experiment' were used to determine D_{max} and K_m by a least squares linear regression fit to equations (4a), (4b), (4c) and (2c). The parameters D_{max} and K_m were also obtained by an iterative computer fit of the same data to the nonlinear equation (3) as well as by a computer program (Cornish-Bowden & Eisenthal 1974) of the DLP technique. In brief, the DLP is a means of graphically determining nonparametric (median) estimates of D_{max} and K_m by extending the lines joining all (negatively assigned) concentration values on the abscissa with their corresponding dose values on the ordinate axis. The median of the intersection points of these lines in the first quadrant has the co-ordinates (K_m, D_{max}) (Eisenthal & Cornish-Bowden 1974; Cornish-Bowden & Eisenthal 1974; Mullen 1977; 1978). (See Fig. 2).

RESULTS AND DISCUSSION

In Fig. 1 the data obtained in an arbitrarily chosen typical 'experiment' (no. 25) are shown along with the 'true' concentration-dose relation calculated from equation (3). Although deviating only moderately from the true data the experimental values in this experiment led to various estimates of the Michaelis-Menten parameters (Table 1) ranging from the low values of K_m = 2·0 mg litre^{-1} and D_{max} = 317·7 mg day^{-1} for equation (4a) to the essentially true values of K_m = 5·0 mg litre^{-1} and D_{max} = 400·6 mg day^{-1} for the iterative computer method based on equation (3). The DLP technique is illustrated in Fig. 2 (a and b) using both the true values (a) and data from 'experiment' number 25 (b). In Fig. 3 (a,b,c,d) it is readily observed that the best linear relationship (r = 0·999) using the data of

Table 1. Values of the Michaelis-Menten parameters relating 'experimental' (computer generated) steady-state serum phenytoin concentrations, C_{ss} and dose, D as determined by various methods. Overall (n = 30) mean (± s.e.m.) values as well as those from a typical single 'experiment' are shown. The 'true' values of K_m and D_{max} are 5·0 mg litre^{-1} and 400 mg day^{-1}, respectively.

Method:	Typical 'experiment' (no. 25)		Mean ± s.e.m. (n = 30 'experiments')	
	K_m (mg litre^{-1})	D_{max} (mg day^{-1})	K_m (mg litre^{-1})	D_{max} (mg day^{-1})
Computer fit to $C_{ss} = \frac{D \cdot K_m}{D_{max} - D}$ (eqn 3)	5·0	400·6	5·0 ± 0·05	400·1 ± 0·3
Direct linear plot	3·5	383·6	4·5 ± 0·25	394·8 ± 1·6
$\frac{1}{C_{ss}} = \frac{D_{max}}{K_m} \cdot \frac{1}{D} - \frac{1}{K_m}$ (eqn 4a)	2·0	317·7	7·0 ± 1·26	478·1 ± 43·5
$\frac{D}{C_{ss}} = -\frac{1}{K_m} \cdot D + \frac{D_{max}}{K_m}$ (eqn 4b)	2·7	358·7	4·3 ± 0·44	390·3 ± 8·3
$C_{ss} = D_{max} \cdot \frac{C_{ss}}{D} - K_m$ (eqn 4c)	4·0	391·5	4·8 ± 0·17	397·5 ± 1·5
$D = -K_m \cdot \frac{D}{C_{ss}} + D_{max}$ (eqn 2c, with v replaced by D)	9·3	341·2	3·4 ± 0·35	354·6 ± 8·0

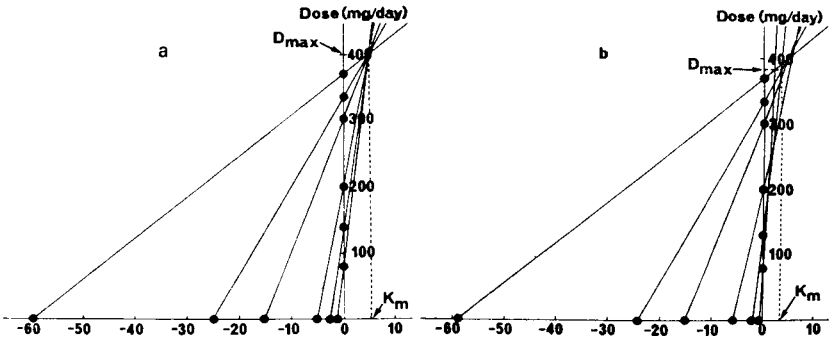

FIG. 2. The direct linear plot of the same concentration (Css abscissa)—dose (Dordinate) data illustrated in Fig. 1. The 'true' or calculated data (part (a)) show a common point of intersection in contrast to the data of 'experiment' 25 (part (b)) in which the median intersection point determines the best estimates of K_m and D_{max}.

experiment 25 is obtained using equation 4(c).

The overall mean (n = 30 'experiments') K_m and D_{max} values obtained by each of the six methods studied are shown in Table 1. Simply on the basis of the absolute difference between the true and experimentally determined mean values, it appears that the reliability of the various techniques for estimating *both* K_m and D_{max} decreases in the following order:

Computer fit > eqn > DLP > eqn > eqn > eqn.
to eqn (3) (4c) (4b) (2c) (4a)

When compared with the other methods investigated, equation (4a) (i.e. 1/C versus 1/D) tended to overestimate both K_m and D_{max} as well as demonstrating by far the greatest variation (coefficient of variation = 98·2 and 49·8% for the mean estimates of K_m and D_{max}, respectively).

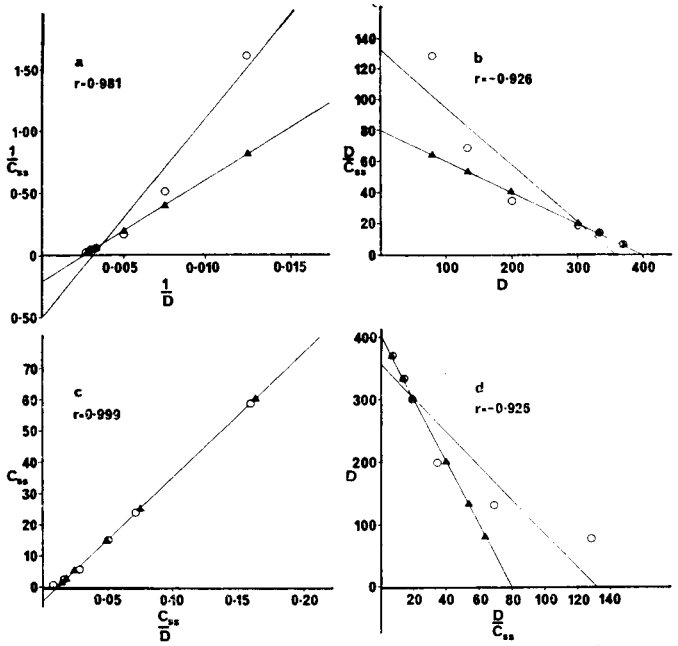

FIG. 3. The various linear graphical techniques based on equations (4a), (4b), (4c) and (2c) are illustrated in parts a,b,c, and d, respectively. Each graph utilizes the 'true' (▲) and typical 'experimental' (○) steady-state concentration (Css)—dose (D) data shown in Fig. 1. The correlation coefficient, r for the regression line through the 'experimental' data points (○) is given for each plot. Equation (4c) appears to provide the best experimental estimates of K_m and D_{max}.

As expected, the present analysis reveals that equation (4c) is superior to other linear transformations (Fig. 3) of the Michaelis-Menten equation in estimating values for the parameters K_m and D_{max} which describe the non-linear relation between serum phenytoin concentrations and dose in the steady-state. At the other extreme, a plot of 1/C versus 1/D (eqn (4a)) was found to be the least reliable means of estimating these parameters. In addition, the results presented in Table 1 indicate that the best linear transformation for use in enzyme kinetics—namely equation (2c) (Dowd & Riggs 1965), should not be employed to determine values of the Michaelis-Menten parameters for the purpose of predicting optimal individualized doses of phenytoin as reported by Ludden et al (1977).

The inferiority of the estimates of K_m and D_{max} obtained from a D versus D/C plot (eqn (2c)) is readily apparent when these values are used in equation (3) to predict the steady-state serum phenytoin concentration on a particular daily dose. Using the mean K_m and D_{max} values (from equation (2c)) listed in Table 1, a 300 mg daily dose of phenytoin would give a predicted steady-state concentration of 18·7 mg litre^{-1} compared with a true value of 15·0 mg litre^{-1}. The concentrations similarly predicted (i.e. calculated using eqn 3) employing the mean Michaelis-Menten parameters determined from equations (4a), (4b), (4c) and the DLP are 11·9, 14·4, 14·8 and 14·3 mg litre^{-1}, respectively. In other words, the results obtained here demonstrate that a plot of D versus D/C, as reported by Ludden et al (1977), may be even slightly inferior to the double reciprocal plot of equation (4a) in predicting specific steady-state concentrations when all experimental error is assumed to be in the measurement of concentration. It is suggested that this peculiar finding may be explained by examining the different D_{max}/K_m ratios (which incidentally, have units of clearance) produced by the various (graphical) methods. The true D_{max}/K_m ratio has a value of 80·0 ml min^{-1} compared with the mean graphically determined ratios which range from the best value of 82·5 ml min^{-1} provided by equation (4c) to 104·3 ml min^{-1} using the parameters obtained by equation (2c)—the corresponding mean value determined from equation (4a) being 67·9 ml min^{-1}.

In the present work 6 data points were used to determine K_m and D_{max} by each method. Since in practice only two or three points would be used initially to estimate the Michaelis-Menten parameters characteristic of an individual patient, it would seem probable that the less reliable methods (i.e. eqns 2(c) and 4(a)) would produce even more spurious predictive results. This study demonstrates that the linear plot of C versus C/D (eqn 4(c)) is superior to other graphical techniques in providing reliable estimates of K_m and D_{max} pertaining to the relation between steady-state serum phenytoin concentration and dose. However, additional studies (data not presented here) have demonstrated that the DLP technique is superior to all the other graphical methods for determining the best estimates of K_m and D_{max} when only one of the six concentration-dose points is replaced by 'error' values of various magnitudes. Thus, due to its simplicity (no calculations) and relative reliability in predicting steady-state phenytoin concentrations on a specific dose (or vice versa, the prediction of the dose required to achieve a desired steady-state concentration) it is reasonable to recommend the previously reported DLP technique (Mullen 1977; 1978) as a reliable clinical aid in the attainment of optimal phenytoin therapy.

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