IDEAL SAMPLING TIME FOR DRUG ASSAYS

P.W. NICHOLSON & S.M. DOBBS

Departments of Physics as Applied to Medicine and of Pharmacology and Therapeutics
The Middlesex Hospital Medical School,
London W1P 7PN

ELAINE M. RODGERS

Department of Pharmacology, Materia Medica and Therapeutics,
University of Manchester, Manchester, M13 9PT

1 Estimation of the mean steady state serum concentration of a drug from an isolated sample apparently requires a knowledge of both the timing of the sample within the dose interval and of the pharmacokinetic constants of the drug in the individual.

2 Data from 80 general medical outpatients receiving maintenance digoxin has been used to derive a relationship between the serum digoxin concentration at any given time (C) and the mean steady state concentration ($\bar{C}$), but this did not allow for individual variation in drug handling.

3 It can be demonstrated theoretically that, for a one compartment model with instantaneous drug distribution and first order elimination, the ratio, $C/\bar{C}$, is dependent on the half time of the drug, the dose interval and the time of sampling. Moreover, for higher values of half time the ratio is virtually constant at 11 h after a daily dose. This, then, would be an ideal sampling time.

4 The above approach can be extended to any drug provided that its half time is at least 15 h in healthy subjects and that the concentration/time curve approximates to a simple exponential decay during virtually the entire dose interval. Orally administered digoxin meets both of these provisions, the ratio at 11 h being 0.97, which is only slightly different from the 1.02 of the theoretical model.

5 Ideal sampling times and the conditions under which they apply may be derived for other dose intervals.

Introduction

Drug assays are being used increasingly despite their expense and demand on laboratory time. However, 'if the timing of the sample is ignored, accurate assessment of further dose requirements is impossible and comparison of results between different laboratories becomes meaningless' (Smith & Rawlins, 1973). During maintenance therapy the mean steady state serum concentration, the area under the serum concentration/time curve divided by the duration of the dose interval, is often the best guide to the average concentration at the site of action. Usually a single blood sample must provide the basis of an estimate of the mean steady state concentration. We consider both the relationship of the concentration at any given time during a dose interval to the mean steady state concentration and the ideal choice of sampling time, with particular reference to digoxin therapy.

Methods and Results

Digoxin data

Eighty consenting general medical outpatients taking maintenance digoxin (36 men and 44 women, aged (mean $\pm$ 1 s.d.) 60 ± 9 years, weighing 62 ± 12 kg and with a creatinine clearance of 66 ± 20 ml/min) were studied. Venous blood samples for digoxin assay were collected just before the daily dose (10.00 h) and 1, 2, 4 and 7 h afterwards. A sixth sample was taken just before the dose on a follow-up visit. Since there was no significant difference in the pre-dose concentration between visits (paired *t*-test, $P > 0.05$) steady state conditions may be assumed.

Digoxin assay

Serum digoxin concentrations were measured by radioimmunoassay (Lanoxitest B, Wellcome Reagents Ltd). The between assay coefficient of variation of a serum sample assayed in duplicate was 10% at a concentration of 1.3 nmol/l and of 2.6 nmol/l.

Characteristics of the serum digoxin concentration/time curves

As a preliminary to further analysis we investigated whether the decay in concentration between 4 and 24 h after the dose was exponential. This was demonstrated by showing the plots of $\log_{10}$ of the concentration at 4, 7 and 24 h against time were linear: the condition for collinearity of the three pairs of co-ordinates (x_1y_1) , (x_2y_2) and (x_3y_3) being that $(y_1 - y_3)(x_3 - x_2) - (y_3 - y_2)(x_1 - x_3) = 0$. This expression, calculated for eighty sets of 4, 7 and (either) 24 h co-ordinates, did not differ significantly from zero ($P > 0.5$).

In order to investigate the relationship of the 1 and 2 h points to the exponential decay, the 1, 2, 4, 7 and 20 h concentrations of every patient were standardized by dividing each by the average of the six. A common regression line was then fitted to the 320 (log standardized concentration, time) co-ordinates, corresponding to the four samples taken 4 h or more after a dose from 80 patients. This line underpredicted the 1 and 2 h standardized concentrations ($P < 0.001$) by a mean of 0.205 and 0.115 log units respectively. Thus an empirical decay curve was constructed: this consisted of a smooth curve drawn through the mean experimental values between 0 and 4 h after the dose and coincided with the regression line for the remainder of the dose interval.

Calculation of individual mean steady state concentrations

The mean steady state concentration achieved by each patient could have been derived from the area under a concentration/time curve constructed from his measured concentrations. However, since the concentrations were not measured at equal time intervals, such an estimate of the mean steady state concentration would depend unduly on the accuracy of the 7 and 24 h measurements. Instead the empirical decay curve described above was fitted to each patient's data so as to minimize the error sum of squares. It was convenient to deal with the concentrations in log units as the fitting process then merely consisted of shifting the empirical curve until the mean of an individual's six log (measured

concentrations) was equalled by the mean of the corresponding log concentrations on the empirical curve. An estimate of a patient's mean steady state concentration equally dependent on the logarithm of each of the six measured concentrations, was thus obtained from the area under his fitted curve.

Relationship of the digoxin concentration at a given time to the mean steady state concentration

The assay results of each patient were expressed as a fraction of his mean steady state concentration. The variation (mean $\pm$ 1 s.d.) in the timing of each sample and in the corresponding ratios are shown in Figure 1. The empirical decay curve was expressed in terms of the ratio, concentration at a given time to mean steady state concentration by the addition of a constant (0.775 $\log_{10}$ units) and is superimposed on the experimental points.

Optimum sampling time

Consider the theoretical case of a drug administered repetitively in a fixed dose of mass M , at time intervals T . The drug is distributed instantaneously in a single compartment of volume V , and eliminated by a first order (exponential) process with a clearance of Cl . The half time $T_{1/2}$ is then given by $(\ln 2)(V/Cl)$.

Once steady state conditions have been attained (i.e. the variation in serum concentration with time is the same during each dose interval), the concentration C at a given time t after the dose is given by

$$C = \frac{M}{V} \left[\frac{\exp(-Cl t/V)}{1 - \exp(-Cl T/V)} \right] \quad (1)$$

and the mean steady state concentration $\bar{C}$ is given by

$$\bar{C} = \frac{M}{Cl T} \quad (2)$$

Thus the ratio $C/\bar{C}$ is given by

$$\begin{aligned} \frac{C}{\bar{C}} &= \frac{Cl T \exp(-Cl t/V)}{V(1 - \exp(-Cl T/V))} \\ &= \frac{0.693T}{T_{1/2}} \left[\frac{\exp(-0.693t/T_{1/2})}{1 - \exp(-0.693T/T_{1/2})} \right] \end{aligned} \quad (3)$$

and can be plotted for different values of the variables, dose interval and half time, as shown in Figure 2. It can be seen that the value of the ratio is virtually constant at 1.02 at 11 h after a daily dose (Figure 2a) and at 5.5 h after a twelve hourly dose (Figure 2b) for wide ranges of half times.

In contrast to the above, digoxin is usually given orally and is slowly distributed from the blood to other tissues. The result is that the serum digoxin concentration/time curve differs from the exponential decay of the theoretical model in the first few hours

Figure 1 Alteration in the ratio, serum digoxin concentration, at a given time/mean steady state concentration ($C/\bar{C}$), with time during a 24 h dose interval. The curve is based on data from a group of patients taking digoxin with a wide range of age, weight and creatinine clearance, and is intended for use in the interpretation of digoxin assay results, when, as is nearly always the case, the half time is unknown.

after the dose (see Figure 1). The area under the individual digoxin concentration/time curves when calculated from all six experimental data points using the trapezoidal method was 1.05 ± 0.06 (mean $\pm$ 1 s.d.) times greater than that estimated from simply extrapolating the regression lines, fitted to each set of 4, 7 and two 24 h log concentration/time points, to the start of the dose interval. Consequently, the mean steady state digoxin concentration achieved would be, on average, only five per cent higher than that estimated by equation (1). We therefore felt justified in adding a scale applicable to daily digoxin to the ordinate of Figure 2a, the ratios for digoxin being given by (expression (3))/1.05). The ratio at 11 h after a daily dose of digoxin can be seen to have a value of 0.97, for a range of half times encompassing all practical values (for which see Avery, 1978).

Discussion

Theoretically measuring serum digoxin concentrations in all patients receiving maintenance therapy would ensure that the dose was both adequate and safe, but is the way that assay facilities are used in clinical practice of any benefit to the patient? Ingelfinger & Goldman (1976) point out that 'because a clinician usually has information on his patient's digitalis dose, renal function, ss, serum potassium and cardiac status, the diagnostic utility of the serum digitalis concentration will depend on whether this test provides information not contained

in these other determinants of the probability of digitalis intoxication.' It is unlikely that this additional information will be provided, unless steady state conditions have been attained, the sampling time with respect to the dose interval recorded, the internal and external quality control of the assay adequate and the doctor has the ability to interpret the report (Dobbs & Dobbs, 1980).

Confirmation that a steady state has been achieved necessitates taking samples on at least two occasions at the same time in the dose interval. Attaining steady state requires not only a sufficient lapse of time but also a compliant patient.

Many laboratories recommend that samples are taken at least 4 to 6 h after the dose, but do not stress that interpretation depends on the actual timing. We have found that in both normal volunteers and patients the peak concentration occurs at a variable time within the first 2 h after a dose and so would agree that sampling before 2 h should be avoided. However, reference to figure 1 shows that if it were not known whether a sample was taken 4 or 24 h after a dose, it would be impossible to differentiate between a potentially toxic concentration and one within the therapeutic range or between a therapeutic and an inadequate concentration. For example, an assay result of 2.2 nmol/l for a sample taken 4 h after a daily dose would represent, according to the empirical curve, a mean steady state concentration of 1.96 nmol/l, whilst for a 24 h sample, it would represent a mean steady state of 2.93 nmol/l. Inspection of the values of the ratio given for the

Figure 2 Ideal sampling time following a maintenance dose. In (a) a 24 h dose interval is considered, in (b) a 12 h interval. It can be seen that the ratio ($C/\bar{C}$) is virtually independent of half time at 11 h after a daily dose and 5.5 h after a 12 hourly dose, for values of $T_{1/2} \geq 15$ h and ≥ 7.5 h respectively. The left hand scale of each ordinate gives the ratios for the theoretical case described. A right hand scale for digoxin is added only to (a) since more frequent administration than daily is rarely necessary.

upper and lower limits of digoxin half time by Figure 2a shows that this interpretation is an over simplification and that a somewhat wider range of

mean steady state concentrations are consistent with the assay result in the example. An attempt to obtain a more accurate estimate of the mean steady state concentration achieved from isolated assay results was made by examining the correlation between the half time for digoxin in individual patients (estimated from the 4, 7 and two 24 h concentrations) and their characteristics (e.g. age, weight, serum creatinine concentration). However, the correlations were not sufficiently strong to be of practical value, probably a reflection of the inherent inaccuracies of such a determination of half time. We, therefore, recommend the use of figure 1 as the best available method of obtaining an estimate of mean steady state concentration from an isolated result and that such an estimate should be included in the assay report.

When the timing of a sample is not dictated by circumstances, inaccuracies in the estimation of mean steady state concentration arising from individual variation in half time can be avoided by taking the sample 11 h after the daily dose, provided the concentration time curve during a dose interval approximates closely to a simple exponential decay with a half time of at least 15 h. This provision was met in the case of oral digoxin, the ratio of the 11 h concentration to the mean steady state being 0.97 for digoxin, only slightly different from that for a simple exponential decay (1.02). An 11 h sample can be obtained during routine working hours by administering the drug at night.

Given the above provisions, the 11 h sampling time will be ideal for other drugs which are administered daily. For dose intervals of less than 24 h, ideal sampling time, and the minimum half time to which it applies, will be in the same proportion to the interval.

Reprint requests should be addressed to Dr S.M. Dobbs, Department of Pharmacology and Therapeutics, The Middlesex Hospital Medical School, London W1P 7PN.

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