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OPTIMAL SAMPLING TIMES FOR MINIMUM VARIANCE OF CLEARANCE DETERMINATION

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1 Clearance of a substance that follows first order kinetics can be determined from multiple plasma concentrations (the standard method) or from only one plasma concentration and an assumed volume of distribution (the simplified method).

2 On the basis of statistical considerations it is shown that the variance of the clearance determined by the standard method has a minimum when the mean sampling time is equal to the reciprocal elimination constant. For the simplified method the variance of the clearance is minimal when the sampling time is equal to the reciprocal elimination constant multiplied by one plus the ratio between the squared coefficients of variation of the concentration and the volume of distribution.

3 The sampling times determined in this way are optimal in the sense that the variance of the clearance estimate will be larger for any alternative choice of the same number of sampling times. In practice, the optimal sampling times can only be determined approximately because the elimination constant is not known exactly. The loss in precision of the clearance estimation arising herefrom will be less if the elimination constant is underestimated than if it is overestimated to the same relative extent.

4 In theory, the simplified method with optimal sampling time will give a more precise clearance determination than the standard method, if the number of optimally spaced sampling times is less than one plus the ratio between the squared coefficients of variation of the concentration and volume of distribution. In studies where each subject serves as his own control and the coefficient of variation of the volume of distribution is relatively small the simplified method will be of higher precision unless the number of samples with the standard method is unusually large.

Introduction

Of the concepts in pharmacokinetics, clearance probably has the greatest potential for clinical applications. It is also considered the most useful parameter for the evaluation of an elimination mechanism, provided that the elimination rate is proportional to concentration. The clearance is usually determined from the plasma decay of a single dose of a substance. The variation of repeated clearance determinations from an individual in a stable situation is at best 5–10%, but is often about 25% (Rowland & Tozer, 1980) and has even been reported as high as 74% (Conney *et al.*, 1979). Every effort must be made to reduce this variation as much as possible.

It was recently shown that the optimum mean time for blood sampling for determination of ^{51}Cr-EDTA clearance is equal to the reciprocal elimination constant, when the optimum mean time was defined as that giving the least change in clearance for a given change in the 'final slope' of the log plasma concentration versus time curve (Bröchner-Mortensen & Rödbro, 1979). The theory of optimal design for parameter estimation in non-linear models has been applied to tracer and pharmacokinetic experiments (Endrennyi, 1981). They have for example shown that the optimal design of a study of monoexponential disappearance is to place half of the measurements as soon as possible and the second half around this time plus the reciprocal elimination constant. These results are, however, not directly applicable in the estimation of clearance.

In this paper we report the results of statistical considerations defining the optimal times of sampling for clearance determinations as those giving the smallest variance and—contrary to the above quoted

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study—without any presumptions on the spacing of the samples. The clearance may be determined on the basis of two or more plasma concentrations measured during the exponential disappearance phase or from a single measurement and an assumed volume of distribution. In both cases there exist sampling times corresponding to clearance estimations with a minimum variance.

Estimation of clearance

General assumptions

The disappearance after bolus administration of a drug or tracer substance from the pool is assumed to follow a first order kinetic model, i.e. the concentration at time t after administration is given by

$$C(t) = (D/V)\exp(-kt), \tag{1}$$

where k is the elimination constant, D is the administered dose, and V is the pool volume. The clearance (CL) is then

$$CL = kV. \tag{2}$$

From measurements of $C(t)$ at various times after administration of the dose it is possible to determine V as well as k by utilisation of the linear relation between $\ln[C(t)]$ and t

$$\ln[C(t)] = \ln(D/V) - kt. \tag{3}$$

The fitting of the straight line to the data points (t_i, Y_i), where $y_i = \ln[C(t_i)]$, may be carried out by a linear regression analysis. This statistical method is known to give unbiased and minimum variance estimates of slope and intercept when y_i are uncorrelated random variables with variance σ^2 and mean values $\ln(D/V) - kt_i$; i = 1,2,...,n. These assumptions about the statistical variation will be maintained in the following. If $\ln[C(t)]$ is normally distributed with variance σ^2 it follows that the coefficient of variation of $C(t)$ is equal to $\sqrt{\exp \sigma^2 - 1}$ which for small values of σ ($\sigma \leq 0.1$) is very close to σ. Thus the standard deviation σ of $\ln[C(t)]$ is virtually equal to the coefficient of variation of $C(t)$.

Analysis of the standard method

By means of $n \geq 2$ observations (t_i, y_i) the estimates of the slope $\hat{k}$ and intercept $\widehat{\ln(D/V)}$ based on linear regression analysis are given as

$$\hat{k} = -\Sigma(y_i - \bar{y})(t_i - \bar{t})/\Sigma(t_i - \bar{t})^2 \tag{4}$$

$$\widehat{\ln(D/V)} = \bar{y} + \hat{k}\bar{t}, \tag{5}$$

where $\bar{y} = \Sigma y_i/n$ and $\bar{t} = \Sigma t_i/n$. All summations are over index i = 1,2,...,n. Hence, the clearance estimate CL is according to (2)

$$CL = D\hat{k}/\exp(\bar{y} + \hat{k}\bar{t}). \tag{6}$$

This method for clearance determination will be denoted the standard method.

Under the statistical assumptions given above it is well known that $\bar{y}$ and $\hat{k}$ are statistically independent with variances σ^2/n and $\sigma^2/\Sigma(t_i - \bar{t})^2$, respectively. Unfortunately, it is not possible to obtain a tractable expression for the distribution of $\widehat{CL}$ or even its mean and variance. It is, however, relatively straightforward to find approximate expressions for the mean and variance by means of a truncated Taylor series expansion of (6) as function of $\hat{k}$ and $\bar{y}$. Using a first order approximation evaluated around the true values of $\hat{k}$ and $\bar{y}$, denoted k and μ, respectively, it follows that

$$\begin{aligned}\widehat{CL} \simeq\ & Dk/\exp(\mu+kt) + [-Dk/\exp(\mu+k\bar{t})](\bar{y}-\mu) \\ & + [D/\exp(\mu+k\bar{t}) - Dk\bar{t}/\exp(\mu+k\bar{t})](\hat{k}-k) \text{ or} \\ \widehat{CL} \simeq\ & \widehat{CL}[1 - (\bar{y}-\mu) + (1/k-\bar{t})(\hat{k}-k)].\end{aligned} \tag{7}$$

Thus the approximate mean $E(\widehat{CL})$ and variance $V(\widehat{CL})$ of the clearance estimate becomes

$$E(\widehat{CL}) \simeq CL, \tag{8}$$

$$V(\widehat{CL}) \simeq \widehat{CL}^2\sigma^2[1/n + (1/k-\bar{t})^2/\Sigma(t_i-\bar{t})^2]. \tag{9}$$

These approximations will improve as $\sigma \to 0$ or as $n \to \infty$ and $\Sigma(t_i-\bar{t})^2 \to \infty$. It is seen that the approximate or asymptotic variance given by (9) has a minimum for $\bar{t} = 1/k$. It is further seen that the coefficient of variation of $\widehat{CL}$ is independent of the mean clearance. Thus the estimation of the clearance with minimal asymptotic variance only requires that the observation times are distributed with mean value $1/k$.

This result may seem surprising because it implies that different distributions of n sampling times with mean $1/k$, which may give quite different variances for $\hat{k}$, will still give the same minimal asymptotic value for $V(\widehat{CL}) = CL^2\sigma^2/n$. To clarify this result, suppose that the estimate $\hat{k}$ deviates randomly from the true value by the factor $1+\epsilon$, i.e. $\hat{k} = (1+\epsilon)k$. According to (5) the estimate of V may be written as $\hat{V} = D/\exp[\bar{y} + (1+\epsilon)k\bar{t}]$. This means that the error on $\hat{k}$ changes the estimates $\hat{V}$ by a factor $\exp(-\epsilon k\bar{t})$. Since $k\bar{t} = 1$ and $\exp(-\epsilon) \simeq 1/(1+\epsilon)$ for small values of ϵ, it follows that the error term cancels out in the product $\widehat{CL} = \hat{k}\hat{V}$. Thus the clearance estimate is insensitive to errors on $\hat{k}$ provided $\bar{t} = 1/k$, and the errors are relatively small.

Figure 1 illustrates how the coefficient of variation of $\widehat{CL}$ divided by σ, i.e. $C(\widehat{CL})/\sigma$, varies as $\bar{t}$ varies around $1/k$. These calculations are based on (9) using $n = 2, 3, 4, 6$ and 10 equidistant sampling times with $t_1 = 0$.

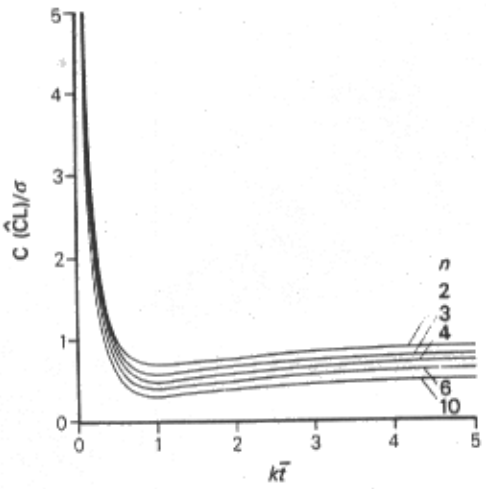

Figure 1 The standard method for clearance estimation of substances based on different values of n equidistantly distributed sampling times. The abscissa is given as the elimination constant k multiplied by the mean sampling time $\bar{t}$. The ordinate is the coefficient of variation of the clearance estimate C($\widehat{CL}$) divided by σ, where σ is the standard deviation of the logarithm of the concentration which is very close to the coefficient of variation of the concentration when σ is small, say < 0.1. All curves exhibit a minimum at $k\bar{t} = 1$.

Analysis of the simplified method

By means of a single measurement of $C(t)$ and an estimate $\widetilde{V}$ of the volume it is possible to determine k from (3) and the clearance estimate becomes

$$\widetilde{CL} = \widetilde{V}[\ln(D/\widetilde{V}) - y]/t. \qquad (10)$$

In (10) y is the natural logarithm of the measured concentration at sampling time t. Hence, y is assumed to be distributed with variance σ^2 and mean $\mu = \ln(D/V) - kt$ as given by (3). $\widetilde{V}$ is regarded as a random variable with mean V and variance ω^2V^2. $\widetilde{V}$ and y are assumed to be uncorrelated. Again it is not possible to derive tractable expressions for the mean and variance of $\widetilde{CL}$. Approximate or asymptotic expressions based on the truncated Taylor series expansion of (10) around (V,μ) can be derived in analogy with equations (7)–(9).

$$\widetilde{CL} \simeq CL + (-V/t)(y-\mu) + \\ + \left[-1/t + [\ln(D/V)-\mu]/t\right](\widetilde{V}-V). \qquad (11)$$

Hence,

$$E(\widetilde{CL}) \simeq CL, \qquad (12)$$

and

$$V(\widetilde{CL}) \simeq CL^2\left[\sigma^2/(kt)^2 + \omega^2[1-1/(kt)]^2\right]. \qquad (13)$$

These approximations will improve as σ^2 and $\omega^2 \to 0$. The variance of $\widetilde{CL}$ shows for positive values of ω^2 a minimum at $t = (1+\sigma^2/\omega^2)/k$. The minimum variance of $\widetilde{CL}$ is $CL^2\sigma^2\omega^2/(\sigma^2+\omega^2)$. When $t \to \infty$, $V(\widetilde{CL}) \to CL^2\omega^2$. If $\omega^2 = 0$, $V(\widetilde{CL}) \to 0$ as $t \to \infty$. If $\sigma^2 << \omega^2$ or $\omega^2 << \sigma^2$ the minimum value of $V(\widetilde{CL}) = CL^2\sigma^2$ or $CL^2\omega^2$, respectively. Here it may seem surprising that the minimum variance of $\widetilde{CL}$ is insensitive to errors on $\widetilde{V}$ if these are relatively small and the coefficient of variation of $C(t)$ is even smaller. Similarly, the minimum variance of $\widetilde{CL}$ is also insensitive to errors on $C(t)$ provided these are relatively small and the coefficient of variation of $\widetilde{V}$ is even smaller. These points may, however, be clarified in the same way as done above for the clearance estimate according to the standard method.

Figure 2 illustrates how the coefficient of variation of $\widetilde{CL}$ relative to σ varies as t varies around $1/k$. These calculations were based on (13) using $\omega^2/\sigma^2 = 0, 0.25, 1, 4$ and 10. It appears that the minimum variance of the clearance becomes more and more sharply defined as ω^2/σ^2 increases, whereas for $0 < \omega^2/\sigma^2 < 1$ the variance is relatively constant around the minimum.

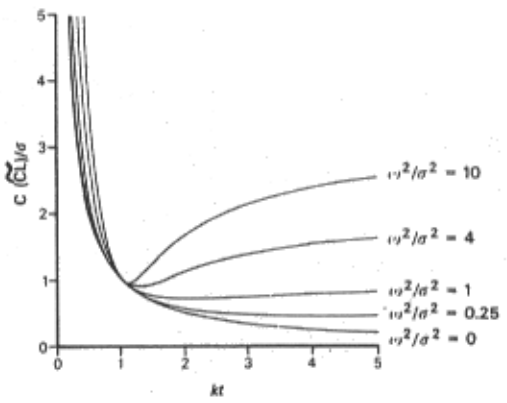

Figure 2 The simplified method for clearance estimation based on one sample of plasma and an assumed volume of distribution. The abscissa is given as the elimination constant k multiplied by the time t at which the sample is obtained. The ordinate is the coefficient of variation of the clearance determination C($\widehat{CL}$) divided by σ, where σ is the standard deviation of the logarithm of the concentration. The curves are drawn for different ratios of ω^2/σ^2, where ω^2 is the squared coefficient of variation of the volume of distribution. When ω^2/σ^2 exceeds 1 the optimal time for the one sample is between $t = 1/k$ and $t = 2/k$. The value of σ is commonly about 0.05 and if the volume of distribution equals the total body water a reasonable value of ω is 0.1. This gives $\omega^2/\sigma^2 = 4$, and the coefficient of variation of the clearance estimate can be found from the corresponding curve in the figure or calculated from equation (13).

Discussion

In this paper it is shown that optimal determination of clearance, in terms of minimizing its variance as a function of the times of sampling, only requires that the mean sampling time is equal to the reciprocal elimination constant. The minimum is independent of how the samples are spaced. These results apply to the standard method based on multiple samples. With the simplified method a minimum also occurs when the sample is obtained at a time equal to the reciprocal elimination constant multiplied by one plus the ratio between the squared coefficients of variation of the concentration and the volume of distribution.

In the calculation of the variances of the clearance we have been obliged to use approximations which, however, will converge to the exact variances when σ and ω decrease towards zero. The accuracy of the approximations has been studied by analysis of the remainder terms of the Taylor series (7) and (11). It was found that the maximum relative error on the approximate variances given by (9) and (13) was about σ^2 or ω^2 provided σ and ω were relatively small (≤ 0.1). Thus the approximations may be considered sufficiently accurate, i.e. within 1% of the exact values, if the coefficients of variation do not exceed 10%. Hence, the existence of the minimum is not an artefact due to the approximations.

The simplified method and the standard method may be compared in the following special case. If the coefficient of variation ω of $\hat{V}$ is equal to σ, this estimate could be regarded as $\hat{V} = D/C(0)$, where D is the dose and $C(0)$ the initial concentration with a coefficient of variation $\sigma = \omega$. This corresponds to the standard method for $n = 2$ and $\bar{t} = t/2$. Since equations (9) and (13) are identical under these conditions, it has been shown that the two methods of clearance estimation in this case are equivalent. The comparison may be further extended to the case of unequal coefficients of variation, σ and ω, for the concentrations $C(0)$ and $C(t)$, respectively. The optimal sampling time is then according to equation (13) equal to $(1+\sigma^2/\omega^2)/k$, and the weighted mean t (of zero and t) is $1/k$ in agreement with equation (9), the weights being $1/\omega^2$ and $1/\sigma^2$, respectively.

These considerations only apply to substances that follow first order kinetics. The results may, however, be extended to substances that follow two or multicompartment models, if the elimination from the compartments differs sufficiently. An example of this is the widely used model drug antipyrine, which has been shown to follow an open two compartment model (Greisen & Andreasen, 1976). The distribution phase is considered completed within one to two hours and the elimination phase due to hepatic metabolism has a half-life of about 12 h in persons with normal liver function (Vestal *et al.*, 1975).

In a considerable number of studies of antipyrine metabolism, where the standard method was applied, the samples were collected within 12 h after drug administration (Ballinger *et al.*, 1972; Døssing & Andreasen, 1981; Elin *et al.*, 1975; Vessel & Page, 1968; Uppal *et al.*, 1980). If, for example, the samples are collected 3, 6, 9 and 12 h after the administration of a drug with a half-life of 12 h, and σ is 5%, then $k\bar{t}$ amounts to 0.43. From equations (8) and (9) it follows that $C(\hat{CL}) = 7.7\%$. In case of a half-life of 24 h and other values as above then $C(\hat{CL}) = 20\%$. If the samples are collected optimally, i.e. spaced around $1/k$, which is 17.3 h in the first example and 34.6 h in the second, then $C(\hat{CL})$ is reduced to 2.5%.

It should be noted that the curves in Figure 1 are calculated on the basis that the first sampling time $t_1 = 0$. This somewhat unrealistic choice was made in order to obtain the lowest possible values for $C(\hat{CL})/\sigma$ with equidistant sampling times when $k\bar{t} \neq 1$ and for comparisons with Figure 2.

Generally, clearance estimation of substances with slow elimination can be determined more precisely and accurately because it is possible to space the samples optimally, respectively avoid sampling in the distribution phase of the substances. From Figures 1 and 2 it is seen that the variance of the clearance increases more steeply from the minimum when $k\bar{t}$ and kt are reduced relative to the optimal value, than is the case when $k\bar{t}$ and kt are increased to the same relative extent. This means that the loss in precision in the clearance estimation will be less if k is underestimated and the sampling times are chosen too large than if k is overestimated and the sampling times are chosen too small. In the latter case there is also an increased risk for systematic errors in the clearance estimate due to incomplete distribution. For these reasons we have recommended an optimum sampling time of 24 h in the antipyrine clearance estimation instead of the calculated optimum time of about 17 h for the simplified method (Døssing *et al.*, 1982). In that study we also showed, empirically, that different ways of estimating the volume of distribution, including a fixed volume of 40 l, only resulted in a slight change in accuracy, i.e. overestimation of the clearance.

Since the minimum variances of the standard method and the simplified method are $CL^2\sigma^2/n$ and $CL^2\sigma^2\omega^2/(\sigma^2+\omega^2)$, respectively, it follows that if $\sigma^2/\omega^2 = n-1$, then the simplified method will provide as precise a determination of clearance as the standard method. If $\sigma^2/\omega^2 < n-1$ the standard method will be more precise than the simplified one, and *vice versa* if $\sigma^2/\omega^2 > n-1$. This is illustrated by the following example. In clinical pharmacology the antipyrine test is widely used to identify and quantitate the effect of drugs and other factors on hepatic antipyrine metabolism (Vesell, 1979). Antipyrine distributes in total body water, which changes very

little during the test period, say ω is about 0.01 for an adult male volunteer. Moreover, with the widely used gas liquid chromatographic method of antipyrine determination σ is about 0.05. Hence, $\sigma^2/\omega^2 = 25$. Accordingly, at least $n-1 = 25$ or $n = 26$ optimally spaced samples are needed with the standard method to give as precise a clearance determination as with the simplified method with optimal sampling time. When studying the effect of factors that may change the volume of distribution, i.e. therapy with steroids, diuretics, etc. or when using fertile women as volunteers, where total body water may change during the menstrual cycle, the standard method may be preferable to the simplified method. In theory, the simplified method should be used if the coefficient of variation of the volume of distribution is low compared to that of the concentration determination. For practical purposes this mainly applies to studies where each subject serves as his/her own control.

In the analysis of the simplified method it was assumed that the volume of distribution was estimated acurately, i.e. the mean value of $\hat{V}$ was equal to the true value V. In practice, depending on the method of determination, this assumption may not be fulfilled. For example, individual volumes determined from a formula based on body weight, height, sex and age will vary around a population mean value, and the coefficient of variation ω will represent an inter-subject variation. This means that the corresponding clearance estimates for given individuals will be more or less biased relative to the true individual values. With respect to the mean clearance of the population the estimates may, however, still be regarded as accurate.

It is concluded that when estimating clearance of a substance there exist optimal sampling times, leading to minimal variance in the clearance determination. During planning and evaluation of investigations consideration should be given to the sampling time(s) in order to minimize this source of variation in clearance estimation.

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