Estimating the clearance of amylobarbitone from a single plasma measurement

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Amylobarbitone sodium (200 mg) was given by intravenous injection to nine healthy, young adults (four males). Subjects were drug-free and nonsmokers. Serial blood samples were drawn for 48 h following the infusion, and multiple sample and single sample estimates of clearance were calculated. The mean ($\pm$ s.d.) values for clearance (CL) and apparent volume of distribution (V) were $0.032 (\pm 0.007) \text{ l h}^{-1} \text{ kg}^{-1}$ and $1.08 (\pm 0.16) \text{ l kg}^{-1}$, respectively. The mean ($\pm$ s.d.) single sample estimate of clearance, $\hat{\text{CL}}$, based on just the 48 h plasma concentrations of amylobarbitone was $0.033 (\pm 0.006) \text{ l h}^{-1} \text{ kg}^{-1}$. The 48 h single sample $\hat{\text{CL}}$ value was shown to reliably reflect the value of CL with little bias and good precision. Values of the 48 h $\hat{\text{CL}}$ when compared to CL exhibited a mean prediction error (mpe) of 1.2% with 95% confidence limits of -6.3% to 9.4% , and a root mean squared error (rmse) of 9.4% . It is concluded that amylobarbitone's clearance can be estimated in a single dose, single sample protocol permitting its use as a single dose, single sample probe for studying host factor influences on drug metabolism.

Keywords amylobarbitone clearance

Introduction

The study of host factor influences on drug disposition is an important facet of clinical pharmacology (Vesell, 1984). Frequently, the investigations of host factor influences on hepatic drug metabolizing activity, especially mixed function oxidase (MFO) activity, employ one of several MFO probes. Of these antipyrine is frequently used, and its application as a probe has been suitably reviewed (Sultatos *et al.*, 1980). Other probes of hepatic metabolism used have included aminopyrine (Hepner & Vesell, 1975), diazepam (Hepner *et al.*, 1977), theophylline, warfarin (Vesell, 1984), and amylobarbitone (Inaba *et al.*, 1976). The widespread testing of host factor influences on drug metabolizing activity is likely to be facilitated if the number of samples required is limited and if samples can be obtained non-invasively. Slattery *et al.* (1980) described a

strategy for estimating individualized maintenance doses from single sample data. Dossing *et al.* (1982) have demonstrated the suitability of a single dose, single sample method for studying host factor influences on antipyrine clearance. Antipyrine clearance can be determined from a single salivary measurement (Sonne *et al.*, 1985). Others have demonstrated that phenytoin (Bachmann *et al.*, 1985a), theophylline (Bachmann *et al.*, 1985b), warfarin (Bachmann, 1986), and ethosuximide (Bachmann *et al.*, 1986) may also be used in single dose, single sample strategies which permit good clearance estimates. Thus they may also be used to probe host factor influences on MFO activity with the administration of single doses and collection of single samples.

In view of amylobarbitone's use as a probe of hepatic drug metabolism in man (Inaba *et al.*,

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1976; Kalow, 1982), its suitability for use in a single dose, single sample strategy for probing drug metabolizing activity was investigated.

Methods

Nine healthy, non-smoking volunteers (four males and five females), aged 21–31 years, and weighing 48.1–75.5 kg (mean, 62.9), participated after giving written, informed consent. Subjects had no history of chronic drug use, took no medications throughout the study period, and abstained from social alcohol consumption beginning at least 48 h prior to the administration of amylobarbitone. Amylobarbitone sodium (Amytal, Lilly) was administered as an intravenous infusion (200 mg in 5% dextrose in water, 100 ml) over 15 min. Whole blood (5 ml) was collected into heparinized tubes from an ante-cubital vein at 0, 0.5, 1, 2, 4, 9, 24, and 48 h after infusion. Samples were centrifuged for 10 min at room temperature on a clinical centrifuge, and the plasma layer was harvested and frozen at -20°C .

Assay

Samples were thawed, and 0.5 ml aliquots were acidified with 50 μl 2 N HCl and extracted with 4 ml benzene: ethylacetate (80:20 v/v) containing secobarbitone as the internal standard. The mixture was rotated for 5 min and centrifuged for 5 min. The solvent layer was transferred and evaporated to dryness under a gentle stream of air. The residue was dissolved in 25 μl CHCl_3 , and 2 μl were injected into a gas chromatograph (Hewlett-Packard 5710A) fitted with a 0.18 m 2510DA column (Supelco) and a flame ionization detector. Column temperature was held at 200°C . Retention times of amylobarbitone and secobarbitone were 0.9 and 1.3 min, respectively. Amylobarbitone concentrations were calculated from peak-height ratios of sample peaks: internal standard peaks using freshly prepared standards. The assay exhibited a coefficient of variation 4.6% at 1.0 mg l^{-1} with a sensitivity limit of 0.3 mg l^{-1} .

Pharmacokinetic calculations

Data were fitted to a linear model using ESTRIP (Brown & Hanno, 1978) operating on a PDP 11/70 computer. Area under the plasma concentration vs. time curve (AUC) was integrated to time, infinity. Total clearance, CL, was calculated as:

$$\text{CL} = \frac{D}{\text{AUC}} \quad (1)$$

where D represents the weight-adjusted dose (mg kg^{-1}). Apparent volume of distribution, V was calculated from:

$$V = \frac{\text{CL}}{\lambda_z} \quad (2)$$

where λ_z is the slow phase plasma concentration disappearance constant.

Single sample clearance estimates, CL, were calculated as described by Dossing *et al.* (1982):

$$\hat{\text{CL}} = \left[\ln \left(\frac{D}{V} \right) - \ln C_t \right] \cdot \frac{V}{t} \quad (3)$$

where C_t is the concentration of amylobarbitone measured in plasma at time, t , and V is fixed at 1.08 l kg^{-1} .

The ability of single sample clearance estimates, $\hat{\text{CL}}$, to predict multisample clearance values was evaluated by estimating mean prediction errors (mpe) and their 95% confidence limits (Sheiner & Beal, 1981).

Simulations

Nine simulated amylobarbitone plasma concentration profiles were generated to examine the robustness of single sample clearance estimates to errors in assumptions above V . For these simulations amylobarbitone was assumed to obey linear, one compartment kinetics. Doses were kept constant at 200 mg (2.86 mg kg^{-1}), but V and CL were varied to plus or minus two standard deviations from their respective means. Clearance estimates, $\hat{\text{CL}}$, based on the 48 h concentrations were compared to the actual CL values for each simulation. The percentage deviation of the single sample $\hat{\text{CL}}$ from actual CL was calculated as $100 \cdot (\hat{\text{CL}} - \text{CL})/\text{CL}$. Simulations were performed using an IBM PC-AT.

Results

Both biphasic and monophasic decay curves were observed. All were apparently linear, and the area under the initial rapid disappearance phase which could be measured contributed negligibly to the entire AUC. Individual values for CL, V , and $\hat{\text{CL}}$ are shown in Table 1. Clearance estimates were calculated based on amylobarbitone measurements at 9 ($\hat{\text{CL}}_9$), 24 ($\hat{\text{CL}}_{24}$) and 48 ($\hat{\text{CL}}_{48}$) h after infusion.

Figure 1 depicts individual plots of $\hat{\text{CL}}$ vs CL for each of the three sampling times. The line shown is the line of identity. It can be seen that

Table 1 Individual values for pharmacokinetic parameters and single sample estimates of clearance based on amylobarbitone concentrations in plasma at 9, 24, or 48 h after the dose.

Subject	Sex	Weight (kg)	V (l kg ⁻¹)	CL*	$\hat{CL}_9^*$	$\hat{CL}_{24}^*$	$\hat{CL}_{48}^*$
MF	F	50.6	1.28	0.038	0.039	0.067	0.044
DL	F	57.8	1.08	0.030	0.039	0.029	0.030
DH	F	75.5	1.36	0.030	0.053	0.036	0.029
DR	F	73.9	1.18	0.039	0.051	0.039	0.038
LS	F	48.1	1.02	0.046	0.041	0.033	0.039
DS	M	66.7	1.02	0.027	0.035	0.025	0.029
JW	M	73.7	0.85	0.027	0.012	0.020	0.030
AM	M	61.7	1.00	0.024	0.027	0.019	0.025
DP	M	58.5	0.97	0.030	0.022	0.026	0.031
Mean		62.9	1.08	0.032	0.035	0.033	0.033
± s.d.		10.2	0.16	0.007	0.013	0.015	0.006

* Clearance values are in units of l h⁻¹ kg⁻¹, and V set at 1.08 l kg⁻¹ is used in the calculation of all CL values.

Figure 1 Single sample amylobarbitone clearance, $\hat{CL}$, vs multiple sample estimates of amylobarbitone clearance, CL . Inset numbers refer to sample times for which amylobarbitone concentrations were used to calculate $\hat{CL}$.

scatter is the least when $\hat{CL}$ is based on a single 48 h measurement. The mean prediction errors and 95% confidence limits at the 9, 24, and 48 h sampling times expressed as percentages of the respective mean multisample clearances were 9.9% (-18, 35); 1% (-27, 28); and 1% (-9, 10). Practically no bias occurs in the single sample estimate of clearance using either the 24 h or 48 h sample, and in accordance with Figure 1, the 95% confidence limits for mpe are smallest when the 48 h sample is used. Root mean squared error is also smallest for the 48 h sample estimate of $\hat{CL}$ with a value of $0.003 \text{ l h}^{-1} \text{ kg}^{-1}$ compared to 0.015 and $0.011 \text{ l h}^{-1} \text{ kg}^{-1}$ for $\hat{CL}_9$ and $\hat{CL}_{24}$, respectively.

Clearance estimates, CL , for amylobarbitone based on 48 h plasma concentrations generated for computer-simulated plasma concentration vs time curves were calculated. Mean values for CL and V and their standard deviations were taken from this panel of subjects. Single sample estimates of clearance deviated from multisample clearance only when the actual V (0.76 l kg^{-1}) was two s.ds. smaller than the assumed value for V (1.08 l kg^{-1}) which is used in equation (3). Even under these circumstances the $\hat{CL}$ value was within 25% of the multisample CL . When the actual V was two s.ds. larger than the assumed value $\hat{CL}$ was within 3–11% of the multisample CL .

Discussion

The suitability of using a single dose, single sample strategy for estimating amylobarbitone clearance has been demonstrated in a panel of nine healthy subjects. Amylobarbitone may therefore prove to be a useful single dose, single sample probe when screening for host factor influences on hepatic drug metabolizing activity. It has already been applied in standard single dose, multiple sample protocols as a probe of mixed function oxidase activity (Inaba *et al.*, 1976). That some subjects exhibited biexponential plasma decay curves while others exhibited monoexponential decay curves bears remark. Balasubramanian *et al.* (1970) demonstrated that amylobarbitone kinetics could be described by a two-compartment model. However, Dvorchik & Vessell (1978) noted that the use of a one-compartment formula to calculate amylobarbitone clearance underestimates clearance by only 8%. The precise and non-biased single sample clearance estimates reported herein tend to reinforce those observations.

The optimal sampling time for estimating

amylobarbitone $\hat{CL}$ when calculated according to Dossing *et al.* (1983) is 37 h based on a coefficient of variation for V of 15% and a coefficient of variation for the assay of 4.6%. Nevertheless, the collection of a sample at 48 h post-dosing seems to provide an excellent estimate of clearance if the assumed V is set at 1.08 l kg^{-1} , the mean value for this panel of subjects. Forty-eight hours is a convenient sampling time when doses are taken in the morning. It should be noted that others have reported very similar values for amylobarbitone's apparent volume of distribution. The value based on data from four male and four female subjects fitted to a two-compartment model was 1.00 l kg^{-1} (Balasubramanian *et al.*, 1970). The value for V calculated from mean λ_z and CL values reported for 36 subjects (Inaba *et al.*, 1976) is 1.17 l kg^{-1} . These investigators reported a mean amylobarbitone CL of $0.034 \text{ l h}^{-1} \text{ kg}^{-1}$. This is similar to the value calculated from the data of Balasubramanian *et al.* (1970) which is $0.035 \text{ l h}^{-1} \text{ kg}^{-1}$. Both the mean ($\pm$ s.d.), multisample clearance of $0.032 (\pm 0.007)$ and $\hat{CL}_{48}$ of $0.033 (\pm 0.006)$ compare favourably to earlier published clearance values.

The use of a single sample estimate of clearance as a tool for screening for host-factor influences on drug metabolism affords the benefit of minimizing discomfort and risks and maximizing convenience to subjects. If each subject is used as his own control in such investigations, then errantly setting a value of V for each subject at a population mean value even for subjects whose actual V deviates substantially from the mean will be virtually inconsequential (Dossing *et al.*, 1972). It is important however, to assure that mean normal population values for V not be errantly invoked among subjects with pathologies who therefore represent a different population relative to V . One must be cautious, moreover not to infer too much from host factor related changes in $\hat{CL}$. For amylobarbitone, host factor induced changes in $\hat{CL}$ will most likely reflect changes in its hepatic metabolism since less than 1% of the intact molecule is excreted in the urine (Inaba *et al.*, 1976). However, though amylobarbitone is extensively metabolized to 3'-hydroxymylobarbitone (Grove & Toseland, 1971), it is also *N*-glucosidated (Clark, 1985). Thus, host factor induced changes in $\hat{CL}$ may provide little insight into the precise metabolic pathway which might be affected.

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