A simple method for determination of antipyrine clearance

Antipyrine clearance (Cl_{AP}) is widely used for assessment of microsomal liver function. The usual procedure involves collection of 4 to 7 samples of plasma or saliva obtained during 24 to 48 hr. To determine whether this procedure could be simplified it was compared with one based on a single sample (sCl_{AP}) and an estimated volume of distribution (V_D) in 142 persons. V_D was estimated from body weight, in kilograms (BW), height, in centimeters (BH), age in years, and sex, or assumed to be 40 l. The agreement between values of Cl_{AP} and sCl_{AP} increased with the time of the single sample and the two clearance estimates were nearly identical in all cases when the sample was taken after 18 hr. The method used for assessment of V_D had only a small influence on the agreement. It is suggested that antipyrine clearance (in ml/min) is estimated as $sCL_{AP} = \frac{\ln(D/V_D) - \ln c_t}{t} \times V_D$, *where D is the dose of antipyrine (in mg), c_t the concentration of antipyrine (in mg/l) at sampling time t (in min), t should be about 1440 min (24 hr), and V_D (in l) is calculated as $0.2363 \times BW + 0.1962 \times BH - 0.0272 \times age - 10.26$ (women) or $0.3625 \times BW + 0.2239 \times BH - 0.1387 \times age - 14.47$ (men). Little information is lost, however, if a fixed volume of 40 l is used. Then, if the dose is 1 gm, c_t is expressed in milligrams per liter, and the sampling time is 24 hr, $sCl_{AP} = (3.28 - \ln c_t) \times 28$ ml/min.*

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Few attempts have been made to define the optimal number and times of sampling blood or saliva for estimation of hepatic elimination rate of model substances in clinical pharmacology and hepatology. The conventional 45-min sampling time used in the Bromsulphalein retention test is mainly empirically founded.[10] Galactose elimination capacity has been measured as 45 and 60 min after galactose administration.[11] It was later shown that reducing the number of blood samples for estimation of galactose elimination capacity leads to large deviations from measurements based on the standard method.[6] A different approach has been used with investigations of single-injection technique of indocyanine green. The indocyanine green concentration in blood 6 min after dosing discriminated better between healthy subjects and subjects with liver disorders than did a method using the half-life (t½) of indocyanine green and based on multiple samples.[12] The aminopyrine breath test was introduced as a single-sample test,[5] but this approach has been questioned by some, who found results based on multiple breath samples more informative.[3,4]

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Table I. *Results of linear regression analyses of Cl_{AP} based on multiple samples and sCl_{AP}*

*Method of V_D prediction**	*Sampling interval (hr after drug administration)*	*No. of samples*	*Correlation coefficient*	*Slope of regression line*	*Intercept of regression line*	*Residual variance (s_e^2)*	*Mean (SD) Cl_{AP} (ml/min)*	*Mean (SD) sCl_{AP} (ml/min)*
Bruce	0-4.5	135	0.80	1.14	4.69	282	30.0 (19.6)	39.3 (28.0)
Hume			0.78	1.14	7.74	317		41.9 (28.5)
Own formula			0.83	1.27	−10.2	293		28.2 (19.6)
40 *l*			0.60	1.36	−11.5	1279		29.4 (44.7)
Bruce	4.51-10.5	139	0.92	0.98	5.19	70.0	30.8 (19.5)	35.4 (20.8)
Hume			0.91	0.98	6.45	75.7		36.6 (20.9)
Own formula			0.93	1.05	−1.90	70.2		30.5 (22.1)
40 *l*			0.78	1.16	−5.03	329		30.7 (28.4)
Bruce	10.51-18	99	0.97	0.94	3.39	76.7	31.4 (16.3)	33.1 (16.1)
Hume			0.97	0.95	3.65	15.3		33.6 (16.0)
Own formula			0.97	1.03	−0.84	15.5		31.6 (17.5)
40 *l*			0.92	1.10	3.01	61.7		31.7 (19.7)
Bruce	18.01-27	137	0.98	0.91	1.37	8.0	28.6 (16.3)	27.6 (15.1)
Hume			0.98	0.91	1.66	7.6		27.5 (15.2)
Own formula			0.98	0.98	−0.63	8.7		27.5 (16.3)
40 *l*			0.97	0.98	−0.65	16.7		27.3 (16.3)
Bruce	27.01-42	102	0.99	0.94	1.34	4.4	25.8 (15.3)	25.8 (14.7)
Hume			0.99	0.94	1.31	4.5		25.8 (14.6)
Own formula			0.99	1.02	0.43	5.1		25.9 (16.7)
40 *l*			0.97	1.01	−0.26	14.3		26.1 (15.9)
Bruce	42.01—	51	0.98	0.91	1.36	4.3	15.1 (9.9)	15.1 (9.2)
Hume			0.97	0.90	1.60	4.9		15.3 (9.2)
Own formula			0.98	0.97	0.59	4.5		15.2 (9.9)
40 *l*			0.97	0.99	0.19	6.9		15.1 (10.2)

*For explanation of methods of prediction, see Material and methods section of text.

Antipyrine clearance (Cl_{AP}) is widely used for quantitative assessment of microsomal liver function.[13] In all studies of antipyrine metabolism, the clearance of the drug is estimated from the plasma or salivary decay based on four to seven samples obtained between 3 and 48 hr after 1 to 2 gm antipyrine by mouth or vein.

Our purpose was to determine whether any information is lost in the measurement of antipyrine elimination when only one sample is used for its calculation.

Material and methods

Cl_{AP} was estimated in 142 patients (77 women and 65 men) during the period 1978 to 1981. The men and women were of comparable age (46.4 ± 18.0 and 41.5 ± 12.6 yr, mean ± SD), but the women were shorter (165 ± 8 cm) and weighed less (61.7 ± 10.9 kg) than the men (176 ± 8 cm and 77.2 ± 15.3 kg). Antipyrine was given by mouth (n = 98) or vein (n = 44) in a dose of 15 mg/kg body weight (BW) and plasma samples of 5 ml were obtained about 3, 6, 12, 24, and 36 hr and sometimes also more than 42 hr after antipyrine. Antipyrine was measured in duplicate by spectrophotometry[1] or gas-liquid chromatography.[9]

Cl_{AP} was calculated as:

$$Cl_{AP} = k \times V_D, \text{ where } k = \frac{dc}{dt} \text{ and } V_D = \frac{D}{c_o}$$

where c is concentration, t is time, k is the elimination constant, estimated as the slope of the linear regression of ln c on time, V_D is the apparent volume of distribution, D is the dose of antipyrine given, and c_o is the extrapolated concentration at zero time. The simplified, one-sample clearance (sCl_{AP}) was in principle calculated in the same way, except that c_o was estimated from an assumed value of V_D and the elimination constant was assessed from c_o and c_t. The equation used was:

$$sCl_{AP} = \frac{\ln(D/V_D) - \ln c_t}{t} \times V_D$$

where t is time of sampling, c_t the corresponding concentration, and other symbols are as above.

Cl_{AP} and sCl_{AP} were calculated in all 142 patients. In each patient sCl_{AP} was determined for six different intervals, separated by periods 4.8, 10.8, 18, 27, and 42 hr after antipyrine dosing, whenever a sample taken within the interval was available. Samples with concentration values below 10.6 μmol/l (2 mg/l) were discarded. For intervals during which several samples had been taken, one was selected at random for the calculations. The assumed total body water (TBW) in liters (equivalent to V_D) was estimated in the four following different ways, where body weight (BW) is measured in kilograms, body height (BH) in centimeters, and age is given in years: (1) From the formula of Bruce et al.[2]:

$$TBW = 0.40 \times BW + 0.23 \times BH - 0.056 \times age + 12.1 \text{ for men}$$

$$TBW = 0.24 \times BW + 0.20 \times BH - 0.03 \times age - 13.9 \text{ for women}$$

(2) From the formula of Hume and Weyers[7]:

$$TBW = 0.296785 \times BW + 0.192786 \times BH - 14.012934 \text{ for men}$$

$$TBW = 0.183809 \times BW + 0.344547 \times BH - 34.270121 \text{ for women}$$

(3) From a multiple linear regression analysis of age, BH, and BW on V_D of the group of women and men from the present material:

$$TBW = 0.3625 \times BW + 0.2239 \times BH - 0.1387 \times age - 14.47 \text{ for men}$$

$$TBW = 0.2363 \times BW + 0.1962 \times BH - 0.0272 \times age - 10.26 \text{ for women}$$

(4) From a fixed V_D of 40 *l* (the mean V_D of the 142 patients calculated from the antipyrine elimination/time curve was 40.8 *l*).

Regression analysis of sCl_{AP} on Cl_{AP} was performed by the least-square method. The values of Cl_{AP} and sCl_{AP} from a given set of data were examined for correlation since the concentration measurment on which the sCl_{AP} is based is also used together with the remaining measurements to calculate Cl_{AP}. This correlation was analyzed by calculation of the correlation coefficient from the approximate variances and the covariance of Cl_{AP} and sCl_{AP} for a large number of combinations (960) covering the range of sample periods, V_Ds, clearances, coefficients of variation of V_D, and measured antipyrine concentrations.[8] These correlation coefficients, due to the interdependence, ranged from 0.01 to 0.30. Accordingly, for high correlations between Cl_{AP} and sCl_{AP}, i.e., r values above 0.90, this interdependence introduces a bias of under 2% ($0.3^2 \times [1 - 0.9^2]$) of r^2. From the regression of sCl_{AP} on Cl_{AP}, it is possible to reveal two kinds of deviation of sCl_{AP} from Cl_{AP}: first, a systemic deviation of sCl_{AP} from Cl_{AP}, as indicated by a deviation of the intercept from zero and a deviation of the slope from one and second, a random deviation between the two estimates as expressed by the residual variance of the regression.

Results

The calculations resulted in one Cl_{AP} for each of the 142 patients corresponding to four, five, or six sCl_{AP}s for each of the four ways of estimating the V_D (Table I). The plots of each set of sCl_{AP}/Cl_{AP} are shown in Fig. 1 and the results of the corresponding regression analysis are summarized in Table I.

When the single sample used for calculation of sCl_{AP} was obtained early, i.e., between 3 and 18 hr after antipyrine dosing, there was systematic deviation of sCl_{AP} from Cl_{AP} so that sCl_{AP} overestimates Cl_{AP}, as indicated by a regression coefficient greater than one. When the sample was obtained more than 18 hr after drug, the regression coefficient approximates unity, indicating close agreement between both clearance calculations. Accordingly, the random variation between the two ways of estimating antipyrine clearance decreases with increasing amount of time before the sample is drawn, as indicated by the rapid decrease of the residual variance (Table I).

Antipyrine elimination was sufficiently slow in only 51 of the 142 patients (15.1 ± 9.9 ml/min) to permit the development of antipyrine concentrations above the detection limit of 10.6 μmol/l (2 mg/l) 42 hr or more after antipyrine dosing (Table I). Correlation and regression analyses of sCl_{AP} on Cl_{AP} in this group revealed that the correlation coefficient

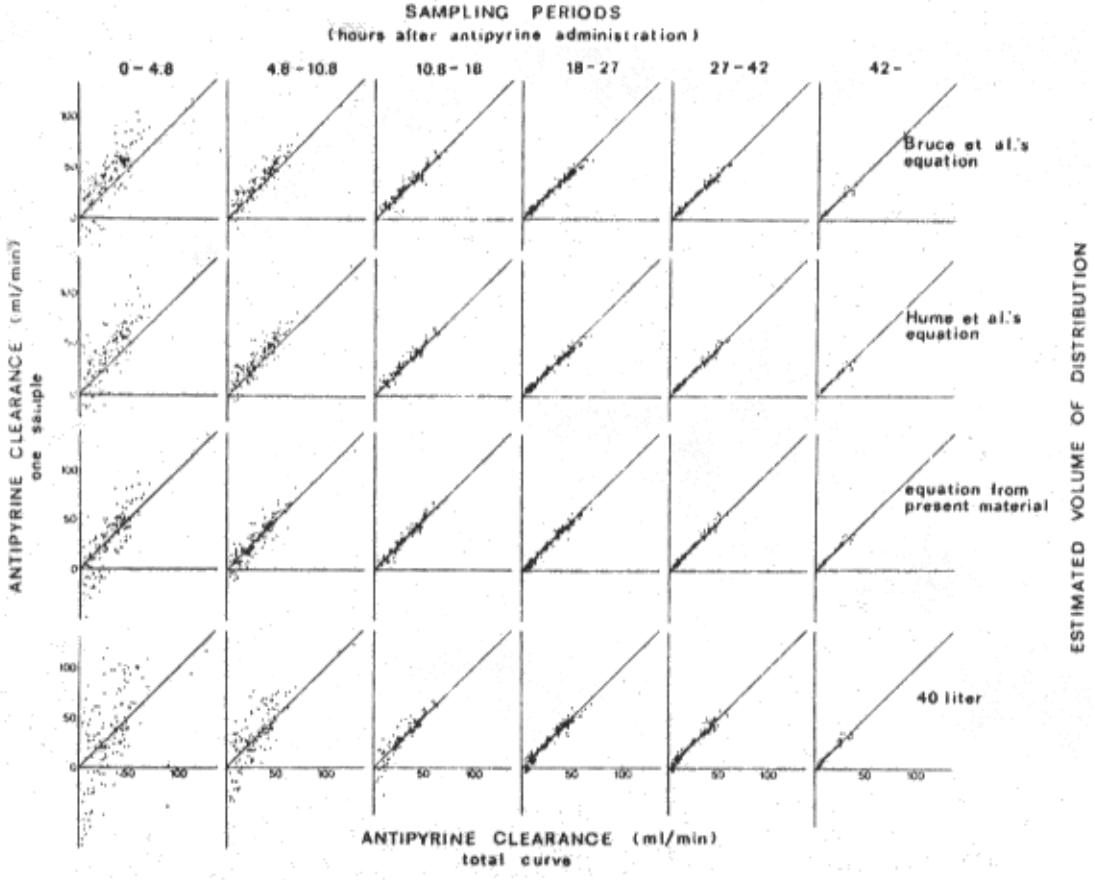

Fig. 1. Plots of corresponding values of antipyrine clearance calculated based on multiple samples and from one sample of plasma. The latter was assessed from plasma samples obtained at five different time intervals after drug dosing and from an estimated V_D.

was highest and the residual variance lowest when the sample used for sCl_{AP} calculation was obtained between 27 and 42 hr after dosing.

Discussion

Our study shows that antipyrine clearance can be estimated from one sample of plasma antipyrine, without systematic deviation from the clearance determinated from multiple samples, and with a very small random variation, provided that the single sample is obtained more than 18 hr after antipyrine. Extension of the sampling interval beyond 42 hr did not improve the agreement between sCl_{AP} and Cl_{AP}. It is therefore concluded that the sample should be obtained at about 24 hr after dosing.

Determination of antipyrine clearance by the one-sample method is simpler than that by multiple samples and almost as reliable. It should, however, be kept in mind that while the multiple-sample method reveals errors (analytical, sampling time, recording, etc.), resulting in a widely deviating point on the concentration-time plot, this is not the case with the singlesample method. The one-sample test is especially applicable in investigations in which each subject serves as his own control and where no alterations in V_D are expected. On the other hand, the test cannot be used in studies in which a measurement of V_D from the antipyrine data is needed.

It is well known that saliva and plasma concentrations of antipyrine correlate closely.[14, 15] The one-sample test can therefore probably be

used noninvasively. In our experience the one-sample saliva test can be carried out by patients themselves with the help of written instructions, so that the test can be used for screening of a large number of patients, including outpatients.

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