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A One-Sample Method for Antipyrine Clearance Determination in Rats

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Abstract. Antipyrine (AP) clearance was determined from AP plasma decay in 57 rats at various times after partial hepatectomy. This value for AP clearance correlated closely (r = 0.99) to AP clearance estimated from a single plasma AP concentration measured 5 h after AP administration. An AP apparent volume of distribution was taken as 0.66 liters per kilogram body weight. The error introduced by estimating AP apparent volume of distribution as 0.66 times body weight was negligible, even when only a single plasma sample for AP concentration served to calculated AP clearance. This single sample method is especially applicable to large scale investigations, acute and chronic, where evaluation of hepatic microsomal function is needed, but time and sampling facilities are limited.

Introduction

Antipyrine (AP) is metabolized primarily by hepatic mixed function oxidases; AP clearance serves widely as a quantitative measure of hepatic function and drug-metabolizing capacity in animals and man [*Vesell,* 1979]. Small rodents are often used in toxicological studies involving determination of hepatic function after liver damage or administration of anticancer drugs [*Capel* et al., 1979; *Wilson* et al., 1982], carbon tetrachloride [*Capel* et al., 1979], polychlorinated biphenyls [*Krampl and Kontsekova,* 1978], phenobarbital and 3-methylcholanthrene [*Danhof* et al., 1979; *Inaba* et al., 1980]. Especially in small rodents a simple, accurate method to assess hepatic function is needed that permits a small sample size and infrequent sampling. Such a method allows experimental designs where changes in microsomal hepatic function can be followed repeatedly in one animal. Recently we described a method to determine AP clearance in humans from a single plasma sample and an AP apparent volume of distribution

[*Døssing* et al., 1982]. The present study was undertaken to investigate the applicability in rats of this simplified method to estimate AP clearance from a single measurement of AP concentration in plasma.

Materials and Methods

Female Wistar rats weighing 177–229 g were fed Rostock® pellets and tap water ad libitum. Either a partial hepatectomy [*Higgins and Anderson*, 1931] or a sham operation consisting of laparotomy, exteriorization, manipulation and replacing of the two anterior liver lobes was performed under diethyl ether anesthesia. Incisions were closed with silk sutures. Surgery was performed between 7 and 11 a.m.

At regular intervals after surgery (3, 6, 12, 24, 72, 96, and 240 h) groups of 6 animals had an injection of 4 mg AP in 1 ml isotonic saline via the tail vein. 6 animals were investigated 3 h after the sham operation. Immediately preceding AP administration, the external jugular vein was cannulated with a silicone catheter (Silastic®) under diethyl ether anesthesia [*Harms and Ojeda*, 1974] for repeated blood sampling. One end was placed in the right atrium of the heart to prevent obstruction of the catheter from coagulation. The other end was brought subcutaneously to pass through the skin at the back of the neck. Rats were housed separately to prevent their biting the catheters.

500 µl blood was drawn into a syringe. Then duplicate samples of 50 µl were also removed after which the 500 µl were reinjected. Samples were drawn approximately 1.6, 2.7, 3.8, 5.0, and 6.3 h after AP administration. Plasma was stored at −20 °C until analysis for AP by high performance liquid chromatography (HPLC). 20 µl of plasma was precipitated with 500 µl methanol-containing phenacetin (10 µg/ml) as internal standard. The supernatant fraction was evaporated to dryness, the residue dissolved in 100 µl mobile phase, and 25 µl was injected into a HPLC-system consisting of a Waters pump and injection loop, a 30 cm Waters microBondapak C18 column and a Waters absorbance detector model 440 with a fixed wavelength of 254 nm. The mobile phase (55/45 methanol/distilled water, v/v) was delivered at a flow rate of 1.5 ml/min.

Calculations

AP clearance (Cl) was calculated as:

$$Cl = K_e \times aVd, \text{ with } K_e = \frac{dc}{dt} \text{ and } aVd = \frac{D}{c_o},$$

where the elimination constant (K_e) is estimated as the slope (dc/dt) of the linear regression of ln (c) on time, aVd is the AP apparent volume of distribution, D is the AP dose given, and c_o is the extrapolated AP concentration at zero time.

The simplified one-sample Cl (Cl-OS) was calculated analogously as:

$$Cl\text{-}OS = K_e \times aVd,$$

$$\text{here } K_e = \frac{\ln(c_o) - \ln(c_t)}{t} = \frac{\ln(D/aVd) - \ln(c_t)}{t},$$

where D/aVd is an estimate of c_o, and aVd is an assumed value of the AP apparent volume of distribution, t is the single sampling time, and c_t the corresponding AP concentration. AP Cl was estimated in 57 rats (6 control animals, 6 sham-operated rats, and 45 rats at different intervals after partial hepatectomy, i.e. 3 (n = 6), 6 (n = 5), 12 (n = 6), 24 (n = 5), 48 (n = 5), 72 (n = 6), 96 (n = 6), and 240 h (n = 6).

For each rat AP Cl-OS was determined at the sampling times t_1–t_5. In 3 cases one sample was lost due to technical difficulties.

Correlations can be artificially elevated due to analysis of data that are not independent [*Oldham*, 1959]. This is the case for the two AP clearance estimates, i.e. the one-sample estimate and the conventional estimate, since they are based in part on identical data. We have shown earlier, however, that this influence is negligible, especially when the correlation coefficient is close to 1 [*Døssing* et al., 1982].

Regression analysis of AP Cl-OS on AP Cl was performed by the least squares method. Slope, intercept, correlation coefficient and residual variation were determined by standard methods. Systematic deviation of AP Cl-OS from Cl, i.e. deviation of the slope from a value of 1, and deviation of intercept from 0, was estimated using the Student's t test. Comparison of several means was performed by one-way analysis of variance. p-values were adjusted for significance and calculated as:

$$p = 1 - [1 - p(t)]^{n-1}, \text{ where } n = 5 \text{ [Duncan, 1955]}.$$

Table I. Results of linear regression analyses of one-sample antipyrine clearance (Cl-OS) on antipyrine clearance determined from total elimination curve (Cl)

	t ($\bar{x}$ ± SD)	n	r[a]	b[a]	p	a[a]	p	s^2
Cl-OS1	1.557±0.370	53	0.893	0.912	0.546	0.035	0.804	0.0105
Cl-OS2	2.756±0.428	56	0.979	1.017	0.958	−0.006	0.993	0.0021
Cl-OS3	3.803±0.433	57	0.981	1.063	0.113	−0.013	0.888	0.0021
Cl-OS4	5.027±0.517	57	0.992	1.022	0.625	0.001	0.999	0.0008
Cl-OS5	6.279±0.872	57	0.984	1.062	0.075	−0.030	0.153	0.0018

t = Sampling times in hours after AP dosing; n = number of samples (rats); r = correlation coefficient; b = slope; a = intercept; p = p-value for a = 0 or b = 1; s^2 = residual variance.

[a] Assumed AP aVd = 0.66 l/kg body weight.

Table II. Animal and pharmacokinetic results

	Mean	SD	Range
Rat body weight, kg	0.1970	0.0121	0.1770 to 0.2290
AP apparent volume of distribution, liters	0.1299	0.0161	0.0982 to 0.1685
AP clearance (all rats), ml/min	0.5103	0.2162	0.2381 to 1.1160
AP clearance (control rats), ml/min	0.7560	0.0812	0.6217 to 0.8268
Elimination constant	−0.2390	0.1073	−0.1047 to −0.5033

Results

Plots of AP Cl-OS on AP Cl for each of the sampling times are shown in figure 1. Corresponding regression analyses are given in table I. The slope of AP Cl-OS on AP Cl at sampling time t_1 (0.912) is less than 1, indicating that AP Cl-OS1 slightly underestimates AP Cl determined by the total elimination curve, but the slope is not statistically significantly different from 1 ($p > 0.05$). Regression analyses of AP Cl-OS on AP Cl at the remaining sampling times also show slopes not statistically different from 1 (i.e., 1.017, 1.063, 1.022 and 1.062, respectively, at t_2–t_5), indicating identity between the two ways of estimating AP Cl. The intercepts are not statistically different from 0. Correlation coefficients are close to 1, except for the regression AP Cl-OS1 on AP Cl.

Residual variance, an expression of the random variation between the two estimates, is small in all regressions, i.e., 0.0105, 0.0021, 0.0021, 0.0008 and 0.0018, respectively, for t_1–t_5.

The assumed AP aVd used for one-sample determination of AP clearance was calculated as 0.66 liter/kg body weight, corresponding to a mean weight of 0.197 kg and to a mean aVd of 0.1299 liters (table II). A plot of the

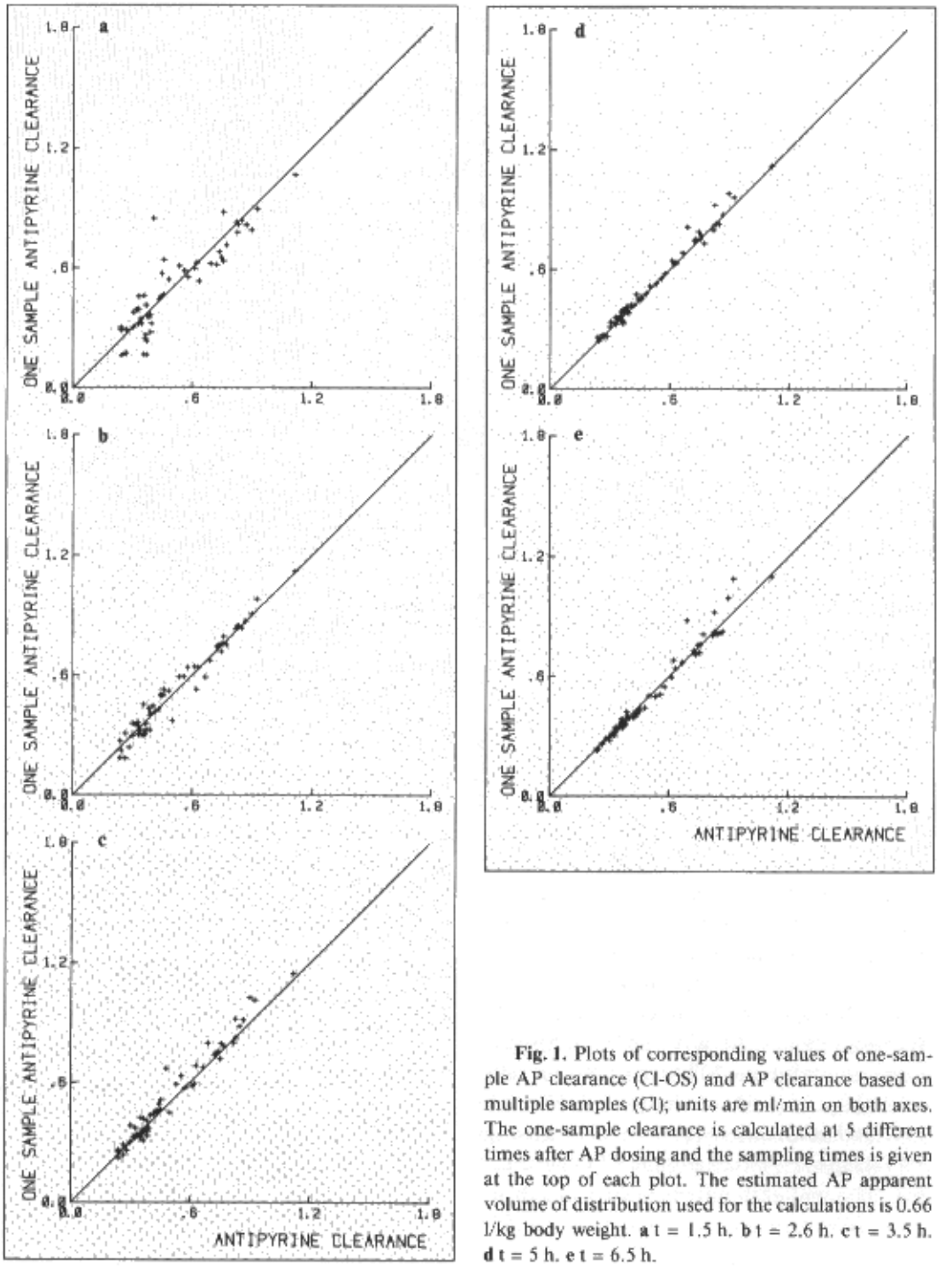

Fig. 1. Plots of corresponding values of one-sample AP clearance (Cl-OS) and AP clearance based on multiple samples (Cl); units are ml/min on both axes. The one-sample clearance is calculated at 5 different times after AP dosing and the sampling times is given at the top of each plot. The estimated AP apparent volume of distribution used for the calculations is 0.66 l/kg body weight. **a** t = 1.5 h. **b** t = 2.6 h. **c** t = 3.5 h. **d** t = 5 h. **e** t = 6.5 h.

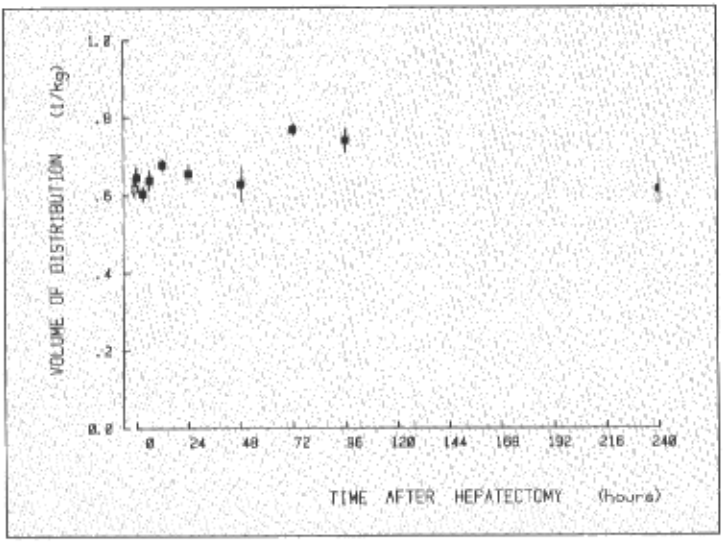

Fig. 2. Plot of AP apparent volume of distribution (liters/kilogram body weight) against the time at which the rats were investigated after hepatectomy. The unfilled dot corresponds to the group of sham-operated rats, and the dot at zero time corresponds to control rats.

AP aVd in liters per kilogram body weight (aVd/BW) determined from the total elimination curve against time after hepatectomy is given in figure 2. Rats investigated 3, 72 and 96 h after hepatectomy vary, respectively, −8.5, +16.7 and +12.4% from the mean (0.66 l/kg), whereas the rest of the investigated groups have values of aVd that are close to this mean value.

Table III shows the influence of biased AP aVd on one-sample AP Cl (Cl-OS4), when the unbiased AP aVd value is assumed to be 0.66 liter/kg. Data are calculated from a body weight of 0.197 kg, a sample time of 5.03 h and a plasma concentration of AP of 9.41 µg/ml. AP Cl-OS4 is rather insensitive to overestimation of AP aVd; up to 20% overestimation (aVd = 0.792 liter/kg BW) results maximally in 1.5% deviation of Cl-OS4. 10% underestimation (aVd = 0.594 liter/kg BW) results in 2% deviation in Cl-OS4 value, and 20% underestimation (aVd = 0.528 liter/kg BW) results in 4.9% deviation in Cl-OS4.

The AP standard curve was linear from 1 to 25 µg/ml AP in plasma. Recovery of AP was 99.4%. The detection limit was 0.05 µg AP/ml plasma, corresponding to a peak-height of 10 mm at the highest sensitivity (0.005 aufs). Relative standard deviation of 10 replicate samples was 2.9% at 4.5 µg AP/ml plasma and relative standard deviation of 45 identical samples analyzed during 6 months was 6.72% at 12 µg AP/ml plasma. No interfering peaks were observed on the chromatograms of precipitated samples. Chromatographic parameters are given in table IV.

Discussion

In this study AP Cl was estimated in rats from one determination of plasma AP concentration, obtained 5 h after AP administration, using an AP apparent volume of distribution assessed as 0.66 liter/kg BW.

Table III. The influence of errors in AP apparent volume of distribution on AP one-sample clearance determination

aVd liter/kg	Deviation of aVd from 0.66 l/kg, %	Cl-OS4 ml/min	Deviation of Cl-OS4 from 0.5104 ml/min, %
0.330	−50	0.4046	−20.7
0.396	−40	0.4383	−14.7
0.462	−30	0.4649	−8.9
0.528	−20	0.4853	−4.9
0.594	−10	0.5002	−2.0
0.627	−5	0.5059	−0.9
0.660		0.5104	
0.693	+5	0.5138	+0.7
0.726	+10	0.5162	+1.1
0.792	+20	0.5181	+1.5
0.858	+30	0.5165	+1.2
0.924	+40	0.5115	+0.2
0.990	+50	0.5034	−1.4

Table IV. Chromatographic parameters

	Antipyrine	Phenacetin
Retention time, tR, min	$tR_1 = 3.2$	$tR_2 = 4.0$
Capacity factor[1], k′	$k'_1 = 0.9$	$k'_2 = 1.4$
Resolution[2], R	1.72	
Relative resolution[3], alpha	1.67	

[1] Calculated as $(tR - t_o)/t_o$, where t_o is the dead time of the column (1.7 min).

[2] Calculated as $2(tR_2 - tR_1)/(w_1 + w_2)$, where w is the baseline width of the peak.

[3] Calculated as $(tR_2 - t_o)/(tR_1 - t_o)$.

The optimal time interval from AP administration to draw the one plasma sample for AP measurement was found to be 5 h, corresponding to a value of 4.9 h predicted according to theoretical considerations [*Døssing* et al., 1983]. The high correlation between the conventional AP Cl estimate and the one-sample estimate indicates that the rough estimate of AP aVd is very accurate and introduces negligible error into calculation of AP Cl from a single plasma AP concentration. To quantitate the influence of such error we recalculated the data for a defined error in the AP aVd. An approach where simulation is performed on genuine data cannot be taken to indicate an exact quantitative solution. However, the error in calculation of the apparent volume of distribution of AP appears to be so small that the one-sample estimate of AP Cl can be considered valid. The one-sample AP clearance is more resistant to an overestimated AP aVd than to one that is underestimated. During liver regeneration AP apparent volume of distribution increases.

The one-sample method to estimate AP Cl results in an estimate with very high variation if the time interval from AP administration to sampling is shorter than the reciprocal elimination constant. Consequently, if the treatment under investigation prolongs AP $t_{½}$, the interval from AP administration to sampling should be prolonged in proportion to the extent of this prolongation [*Døssing* et al., 1983]. This change in sampling time can be determined accurately during a single trial run performed expressly for this purpose. Then the appropriate interval can be selected for subsequent experiments.

We suggest that the simplified sample method be used for large scale investigations, acute and chronic, where estimation of he-

patic microsomal function is needed. It has been shown that saliva can be obtained from small rodents [*Wilson* et al., 1982]. Since saliva and plasma AP concentrations are very closely correlated and often identical [*Vesell* et al., 1975; *Fraser* et al., 1976], the one-sample method can be performed noninvasively allowing repeated estimations in the same animal.

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References

Aarbakke, J.: Impaired oxidation of antipyrine in stagnant loop rats. Scand. J. Gastroent. *12:* 929–935 (1977).

Capel, I.D.; Jenner, M.; Dorrell, H.M.; Williams, D.C.: Hepatic function assessed (in rats) during chemotherapy with some anticancer drugs. Clin. Chem. *25:* 1381–1383 (1979).

Danhof, M.; Krom, D.P.; Breimer, D.D.: Studies on the different metabolic pathways of antipyrine in rats: influence of phenobarbital and 3-methylcholanthrene treatment. Xenobiotica *9:* 675–702 (1979).

Duncan, D.B.: Multiple range and multiple F tests. Biometrics *11:* 1–42 (1955).

Døssing, M.; Poulsen, H.E.; Andreasen, P.B.; Tygstrup, N.: A simple method for determination of antipyrine clearance. Clin. Pharmacol. Ther. *32:* 392–396 (1982).

Døssing, M.; Vølund, Å.; Poulsen, H.E.: Optimal sampling times for minimum variance of clearance determination. Br. J. clin. Pharmacol. *15:* 231–235 (1983).

Fraser, H.S.; Mucklow, J.C.; Murray, S.; Davies, D.S.: Assessment of antipyrine kinetics by measurement in saliva. Br. J. clin. Pharmacol. *3:* 321–325 (1976).

Harms, P.G.; Ojeda, S.R.: A rapid and simple procedure for chronic cannulation of the rat jugular vein. J. appl. Physiol. *36:* 391–392 (1974).

Higgins, G.M.; Anderson, R.M.: Experimental pathology of the liver. 1. Restoration of the liver of the white rat following partial surgical removal. Archs Path. *12:* 186–202 (1931).

Inaba, T.; Lucassen, M.; Kalow, W.: Antipyrine metabolism in the rat by three hepatic monooxygenases. Life Sci. *26:* 1977–1983 (1980).

Krampl, V.; Kontsekova, M.: Effect of polychlorinated biphenyls on the elimination rate of antipyrine from plasma of rats and man. Bull. env. Contam. Toxicol. *20:* 191–198 (1978).

Oldham, P.D.: A note on the analysis of repeated measurement on the same subject. J. chron. Dis. *15:* 969–977 (1959).

Vesell, E.S.: The antipyrine test in clinical pharmacology: conceptions and misconceptions. Clin. Pharmacol. Ther. *16:* 275–286 (1979).

Vesell, E.S.; Passananti, G.T.; Glenwright, P.A.; Dvorchik, B.H.: Studies on the disposition of antipyrine, aminopyrine and phenacetin using plasma, saliva and urine. Clin. Pharmacol. Ther. *18:* 259–272 (1975).

Wilson, V.L.; Larson, R.E.; Moldowan, M.: A noninvasive method for the study of hepatic drug metabolism in rodents. Antineoplastic drug effects on antipyrine metabolism in mice. Chem. biol. Interactions *40:* 159–168 (1982).

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