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Journal of Pharmaceutical and Biomedical Analysis
15 (1996) 7–11

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

# Accuracy of the one-sample method for determination of antipyrine clearance in elderly subjects

F. Jorquera[a], M.M. Almar[b], M. González-Sastre[a], I. Suarez[c], J. González-Gallego[b,*]

[a]*Research Unit, Hospital de Insalud, León, Spain*
[b]*Department of Physiology, Pharmacology and Toxicology, University of León, Campus Universitario, 24071 León, Spain*
[c]*Santa Luisa Residence, León, Spain*

Received for review 16 October 1995; revised manuscript received 29 February 1996

**Abstract**

The purpose of this study was to evaluate the validity of the one-sample abbreviated method for determination of the pharmacokinetic parameters of antipyrine in the elderly. Antipyrine pharmacokinetics were studied in 15 elderly women (mean age 86 years). Antipyrine (1 g) was administered orally and pharmacokinetic parameters were determined by the one-sample (24 h) and multiple-sample (3, 6, 9, 12 and 24 h) methods. Mean antipyrine clearance for the one-sample study ($19.72 \pm 1.51$) was almost identical to that obtained with the multiple-sample approach ($20.73 \pm 1.57$), and the two methods were very well correlated ($r = 0.989$). Relative standard deviations between individual clearances values for multiple-sample vs. one-sample studies averaged 1.6%. Values of elimination half-life were likewise very similar for the abbreviated ($17.41 \pm 1.21$) and complete ($17.99 \pm 1.09$) methods, with a significant correlation ($r = 0.857$). Although values were underestimated by 10% in the one-sample approach, no difference in the volume of distribution with the multiple-sample study was observed. When the unbiased volume of distribution value was determined from the total elimination curve against time, the influence of biased volume of distribution resulted in a 5.1% deviation in antipyrine clearance in the one sample method. The findings indicate that antipyrine pharmacokinetic parameters can be estimated with reasonable precision and accuracy in the elderly using a simplified one-sample procedure.

*Keywords:* Antipyrine clearance; Elderly patients; One-sample method

## 1. Introduction

Measurement of antipyrine clearance is a widely used method for assessing drug metabolizing capacity. This drug has excellent absorption and is extensively metabolized by the cytochrome P-450 liver enzymes. Antipyrine shows a negligible protein binding and its elimination is not limited by liver blood flow, which declines with age. Changes in hepatic oxidative capacity contribute to altered responses to drugs in the elderly, and specific information concerning hepatic function can be obtained by estimation of antipyrine kinetic parameters [1]. In a number of studies

* Corresponding author. Tel.: (+34) 87-291258; fax: (+34) 87-291267.


*PII* S0731-7085(96)01822-5

lower clearances and longer half-lives of antipyrine have been reported in elderly subjects compared to younger subjects [2–9].

Estimation of pharmacokinetic parameters of antipyrine has traditionally required several blood or saliva samples during the 48 h following administration of a single dose of the drug. The difficulty of obtaining serial samples has led to the development of less extensive sampling procedures that enhance the utility of antipyrine studies [10–12]. It is known that antipyrine elimination can be estimated with reasonable precision using two-point sampling procedures [13]. A single specimen method for calculating antipyrine clearance in subjects of middle age, published and validated by Dossing and co-workers [10,14], has veen frequently used as an indicator of drug oxidative metabolism in humans [1]. The validity of the one-sample estimation has been studied in children [15]. However, this method has not been validated in older subjects who, due to changes in salivary flow, intercurrent diseases and ingestion of additional drugs [6], may not provide 24 h antipyrine concentrations as reliable as those of the middle age subjects used by Dossing and co-workers [10,14].

The purpose of this study was to validate the applicability of the one-sample approach for determination of antipyrine clearance in the elderly. Antipyrine pharmacokinetic parameters were estimated by both the one-sample simplified approach and the conventional test based on multiple saliva sampling.

## 2. Experimental

### 2.1. Subjects and procedures

Antipyrine pharmacokinetic parameters were measured in a group of 15 randomly selected old women from a nursing home. Their mean age was 86 years; the age range was 78–95 years. Subjects gave informed consent before entering the study. Inclusion required that they be medically stable, with no hospitalizations within the month before the study. All subjects were free from any acute or chronic hepatic or renal disease as well as from metabolic disorders. Antipyrine (1 g) was administered orally. Saliva samples were collected at 3, 6, 9, 12 and 24 h following antipyrine administration.

### 2.2. Methods

Antipyrine concentration was determined by a high-performance liquid chromatography (HPLC) technique [16,17] as follows: 100 $\mu$l of phenacetin, used as the internal standard, was added to 1 ml saliva. The HPLC system consisted of a SP8000 pump (Spectra Physics), a Spheri-10 RP-18 10 $\mu$ column (Brownlee™ Columns), and a Spectra Chrom 200 detector (Spectra Physics) set at 254 nm. Column temperature was controlled at 40°C by a water circulator. The mobile phase consisting of 0.1 M sodium acetate, 7.5% acetonitrile and 0.5% TEA, pH 6.6, was delivered at a flow rate of 3.5 ml $min^{-1}$.

Kinetic variables for antipyrine based on the multiple-sample method were calculated from the logarithm of the saliva concentration vs. time curve using the following equation:

$$Cl_{AP} = K_e \times V_d$$

with $K_e = dc/dt$ and $V_d = D/C_0$, where the elimination constant ($K_e$) is estimated as the slope ($dc/dt$) of the linear regression of $\ln(c)$ with time and $C_0$ is the extrapolated antipyrine concentration at zero time. $Cl_{AP}$ is the antipyrine clearance. The simplified one-sample antipyrine clearance and half-life were calculated by the equations [10]:

$$Cl_{AP} = \frac{\ln(D/V_d) - \ln C_t}{t} \times V_d \quad t_{\frac{1}{2}} = \frac{0.693 V_d}{Cl_{AP}}$$

where $D$ is the dose of antipyrine given, $V_d$ is the apparent volume of distribution, $t$ is the time of sampling (24 h) and $C_t$ is the corresponding concentration. $V_d$ was calculated in the one-sample method from a multiple regression analysis of age, body weight (BW) and height (BH), according to the following formula [10]:

$$V_d = (0.2363 \times BW) + (0.1962 \times BH) - (0.0272 \times age) - 10.26$$

### 2.3. Statistical methods

The significance of the differences between means was determined by the Mann–Whitney $U$ test. Regression lines were fitted by least-squares regression analysis. Correlation coefficient ($r$) and residual variation ($s^2$) were determined by standard methods. Relative standard deviations (RSDs) between pairs of individual values were determined as the standard deviation (SD) divided by the mean, expressed in percent. Analyses were run with the SPSS for Windows statistical package, version 5.0.2. (SPSS Federal Systems, Chicago, IL).

## 3. Results

Although values were underestimated by 10% in the simplified approach, no difference in $V_d$ with the conventional approach was observed (Table 1). When the unbiased $V_d$ value was determined from the total elimination curve against time, the influence of biased $V_d$ resulted in a 5.1% deviation in $Cl_{AP}$ in the one-sample method. The $Cl_{AP}$ value and antipyrine half-life estimated by both methods were almost identical (Table 1). A linear regression analysis of the estimated $V_d$ in the one-sample method against the measured $V_d$ from the total elimination curve against time (Fig. 1) gave a correlation coefficient, curve slope and intercept indicating no systematic deviation. Plots of one-sample $Cl_{AP}$ and half-life against corresponding values from the multiple-sample study are shown in Figs. 2 and 3. Correlations were also high and no significant systematic deviations were recorded. Residual variance, an expression of the

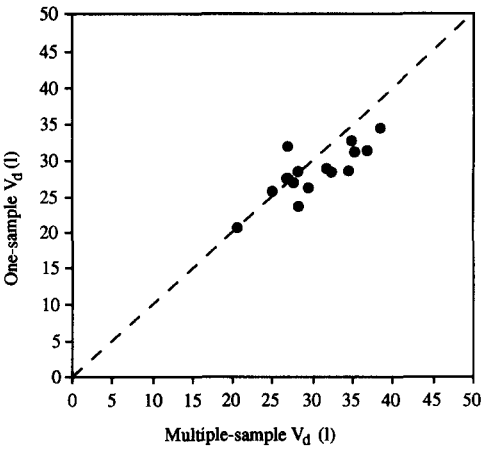


Fig. 1. Relationship between results of antipyrine volume of distribution ($V_d$) calculated from the one-sample method and those from the multiple-sample study. $y = 0.524x + 11.707$; $r = 0.795$; $p < 0.001$; $s^2 = 4.128$. Broken line is the line of identiy ($y = x$).

Table 1
Comparison of kinetic parameters for the one-sample and multiple-sample studies

| Parameter | $V_d$(l) | $t_{1/2}$(h) | $Cl_{AP}$ (ml min$^{-1}$) |
|---|---|---|---|
| Multiple sample | | | |
| Mean ± SEM | 30.62 ± 1.26 | 17.99 ± 1.09 | 20.73 ± 1.57 |
| ± SD | 4.89 | 4.24 | 6.08 |
| Range | 20.79–38.56 | 11.00–26.66 | 13.44–30.47 |
| Median | 29.02 | 16.91 | 18.94 |
| One sample | | | |
| Mean ± SEM | 27.76 ± 0.83 | 17.41 ± 1.21 | 19.72 ± 1.51 |
| ± SD | 3.22 | 4.71 | 5.88 |
| Range | 21.11–33.62 | 10.26–26.74 | 11.38–29.34 |
| Median | 27.59 | 16.65 | 18.68 |
| RSD (%)[a] | 5.60 | 5.13 | 3.78 |

[a] Overall mean values of all individual RSDs for multiple-sample vs. one-sample study.

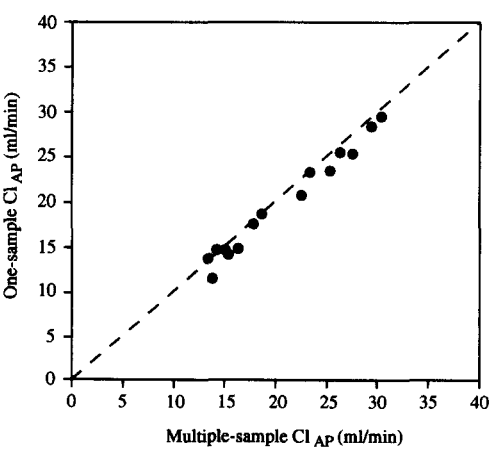


Fig. 2. Relationship between results of antipyrine clearance ($Cl_{AP}$) calculated from the one-sample method and those from the multiple-sample study. $y = 0.955x - 0.078$; $r = 0.989$; $p < 0.001$; $s^2 = 0.844$. Broken line is the line of identiy ($y = x$).

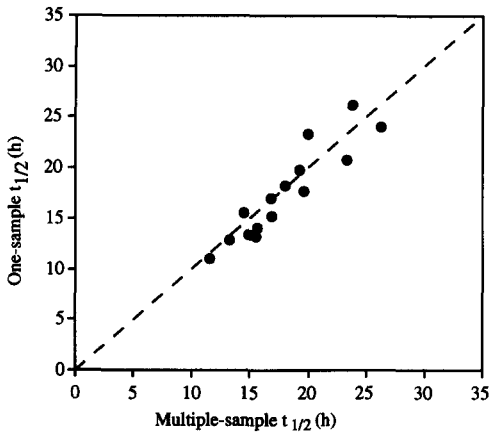


Fig. 3. Relationship between results of antipyrine half-life ($t_{1/2}$) calculated from the one-sample method and those from the multiple-sample study. $y = 0.952x + 0.290$; $r = 0.857$; $p < 0.001$; $s^2 = 6.382$. Broken line is the line of identity ($y = x$).

random variation between two estimates, was small in all regressions (Figs. 1–3).

When the validity of the formula for estimating $V_d$ in the one-sample method was examined in terms of mean error and root mean square error, as estimates of precision and the bias, values of 2.86 and 2.03 respectively were obtained. The evaluation of the distribution of individual RSD values (Table 2) indicated that RSDs vs. the multiple-sample approach rarely exceeded 10% for the abbreviated method in any of the pharmacokinetic parameters. In particular, clearance values using the one-sample method were within 10% of the reference values in 93% of subjects.

Table 2
Distribution of RSDs of antipyrine pharmacokinetic parameters

| Parameter | Fractions of all RSDs (vs. multiple-sample method) | | |
|---|---|---|---|
| | <5.0% | 5.0–10.0% | 10.0–15.0% |
| $V_d$ | 0.40 | 0.46 | 0.14 |
| $Cl_{AP}$ | 0.80 | 0.13 | 0.07 |
| $t_{1/2}$ | 0.46 | 0.40 | 0.14 |

## 4. Discussion

In this study, saliva concentrations were used to characterize antipyrine elimination because, irrespective of the rate of secretion and pH, saliva maintains antipyrine concentrations close to those in plasma, providing clearance values that may be used interchangeably [4,18].

Quantitative assessment of liver function by antipyrine clearance has traditionally required collection of several blood or saliva samples during the 24–48 h following administration of a single dose of the drug. Multiple sampling procedurers are unpleasant for the subjects and occasionally inconvenient for investigators, imposing severe limitations on the utility of the antipyrine test. The development of HPLC analytical methods for antipyrine have greatly improved the sensitivity and specificity of antipyrine measurements [19], allowing satisfactory calculations with a smaller number of samples.

Different abbreviated antipyrine tests have been described. McPherson et al. [11] reported semiquantitative data with a two-sample (4 h and 24 h) clearance test, although the use of a gas chromatographic antipyrine assay imposed a limitation on the utility of the method. Farrell and Zaluzny [12] have also described a two-point method for the calculation of antipyrine pharmacokinetic parameters that appears to be particularly useful as a dynamic test that can be performed repeatedly in patients with liver diseases. Dossing and co-workers [10,14] have shown that antipyrine clearance can be calculated from a single sample. The one-sample method requires that the volume of distribution be calculated from equations relating total body water to age, sex and height and does not allow detection of single errors in sampling time or analysis. However, this method has been demonstrated to be minimally affected by changes in the volume of distribution and avoids the systematic deviation of the method based on the collection of multiple samples [1,20].

Aging is associated with a reduction in antipyrine clearance and a significant increase in antipyrine half-life [2–9]. This may be a primary change in the elderly, although different factors affecting drug metabolism could also contribute

to the decline of antipyrine elimination with advancing age. The apparent volume of distribution is significantly reduced with age, probably reflecting the reduced proportion of lean body weight relative to total body weight in the elderly [2]. Due to the elevated incidence of certain chronic diseases and the increased prescription of drugs with the elderly there is a need for sensitive and abbreviated methods that can be used for longitudinal studies and for screening of a large number of patients, including outpatients, facilitating research into drug oxidative metabolism in the elderly.

The present study shows that estimation of antipyrine clearance from one sample of saliva provides data as accurate as the conventional multiple-sample method, with very small random variation. The high correlation between the conventional antipyrine clearance method and the one-sample approach and the values for the estimates of precision and the bias indicate that the calculation of the apparent volume of distribution from standard equations introduces no significant error in the determination of antipyrine clearance from a single saliva sample. The validity of the single specimen method for calculating antipyrine clearance previously established in middle-age adults [10,14] and children [3] may thus be extended to elderly subjects.

In summary, the findings of this study indicate that antipyrine pharmacokinetic parameters can be estimated with reasonable precision in the elderly using a simplified one-sample approach. It has recently been reported [6] that medically stable elderly subjects show a high reproducibility of individual rates of antipyrine metabolism. Given the safety and usefulness of antipyrine for evaluation of oxidative metabolism, the current findings support the use of the one-sample antipyrine test for treatment planning and for clinical evaluation of the effects of changes in drug regimen or medical condition in the elderly.

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