Plasma antipyrine half-life can be determined from urine data

JOSHUA O. ATIBA*, GLYN TAYLOR, ROBERT A. PERSHE & TERRENCE F. BLASCHKE
Division of Clinical Pharmacology, Department of Medicine, Stanford University School of Medicine, Stanford, California, USA and The Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cardiff, UK

1 Antipyrine half-life has been determined from measurements of antipyrine concentrations in spontaneously voided urine specimens in eleven subjects, studied on a total of forty-seven different occasions while receiving no drugs, interferon or ketoconazole.
2 Plasma and saliva half-lives show good intrasubject correlation.
3 Plasma and urine half-lives show good intrasubject correlation provided total urine output is at least 1.1 l day\(^{-1}\). The range of intrasubject correlation coefficients for plasma and urinary half-lives was 0.76 to 0.98, with a median value of 0.85.
4 Saliva and urine half-lives show good intrasubject correlation, with the range of intrasubject correlation coefficients from 0.74 to 0.98, and with a median value of 0.75.
5 There is a small but consistent bias towards shorter urinary half-life estimates; this averaged 0.75 h for the plasma-urine studies and 0.192 h for the saliva-urine studies.
6 There were parallel changes in antipyrine half-life estimated from plasma and urine for one of our subjects who received multiple doses of recombinant \(\beta\)-interferon and had a 150% increase in antipyrine half-life over the study period.

Keywords antipyrine half-life plasma urine saliva

Introduction

Antipyrine has been widely used as a model drug for studying hepatic drug metabolism (Vesell & Penno, 1983). Antipyrine is distributed throughout total body water with negligible binding to tissue or plasma proteins, and has a low hepatic extraction ratio. Changes in antipyrine half-life in plasma generally reflect changes in hepatic antipyrine metabolism (Sultatos et al., 1980). A number of reports have shown the validity of using salivary antipyrine concentrations as an indirect measure of plasma antipyrine kinetics (Vesell et al., 1975; Welch et al., 1975; Fraser et al., 1976; Danhof & Breimer, 1979; Toverud et al., 1981; Eichelbaum et al., 1983). In a preliminary report, using two healthy volunteers who were not receiving drugs, we demonstrated that the urinary concentration of antipyrine was independent of flow, and that antipyrine half-life could be determined directly by measuring urinary concentrations of antipyrine in spontaneously voided samples (Taylor & Blaschke, 1984). In this report we demonstrate the utility of this approach in a heterogenous group of subjects by correlating antipyrine half-lives estimated from conventional approaches (measurement of plasma and saliva concentrations) with simultaneous estimates derived from urinary concentrations.

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*Present address: CCA of British Columbia, 600 W. 10th Avenue, Vancouver, B.C., Canada V52 4E6
Methods

Subjects

Eleven subjects were studied on a total of forty-seven different occasions. There were six males and five females ranging in age from 25–66 years and weighing 65–100 kg. All subjects gave written informed consent and all protocols under which the subjects were studied had been approved by the Stanford University Committee for Protection of Human Subjects in Research. These individuals were either normal subjects participating in a study of the effect of ketoconazole on hepatic clearance, or cancer patients involved in a study of interferon efficacy and toxicity.

Clearance studies

After an overnight fast, subjects were given 1 g of antipyrine dissolved in about 200 ml of water. Blood samples were drawn into heparinized tubes prior to drug administration and at approximately 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 32 h after drug administration. Plasma was separated and stored at −20°C until analysis. Mixed saliva samples were collected at about the same times as blood samples by having subjects chew on Parafilm® and expectorate into a glass vial. Spontaneously voided urines were collected, the collection interval and volume voided were recorded, and an aliquot stored in a glass vial at −20°C until analysis. The duration of urine collection was from 24 to 48 h, the volume collected was from 1.1–7.8 l, and the urine flow rate ranged from 0.5 ml min⁻¹ to 9.2 ml min⁻¹. The urine collection intervals ranged from 0.5–8 h, and the total number of specimens collected ranged from 5 to 20 per subject. Antipyrine concentrations in plasma, urine and saliva were measured by h.p.l.c. (Danhof et al., 1979).

Data analysis/calculation

A monoexponential function was used to describe the post-peak plasma and saliva concentrations. An extended least squares non-linear regression program, ELSFIT (Sheiner, 1981) was used for this analysis. Urinary concentration data were also described by a monoexponential function using the time of the midpoint of the collection interval as the independent variable.

The half-lives determined from plasma, urine, and saliva data were compared by correlation analysis. Of forty-seven individual studies, there were sixty-six combination studies, made up of thirty-five plasma-urine and thirty-one saliva-urine studies.

On reviewing the data, subjects with urine outputs less than 1.1 l day⁻¹ tended to have a large (greater than 15%) coefficient of variation of the estimate of the elimination rate constant derived from urine data, and a poor linear correlation of urine and plasma half-lives, and were thus excluded from the final analysis. Thus, out of sixty-six antipyrine combination studies, ten (two plasma-urine and eight saliva-urine studies) were excluded on the above grounds.

Results

The results of analysis of the two series of antipyrine combination studies are shown in Figure 1 (plasma-urine) and Figure 2 (saliva-urine). Analysis of variance shows that there is no evidence of an intersubject variance component, so the 33 observations from the former and the 23 studies in the latter can be legitimately treated as one large sample. For the plasma-urine study

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Figure 1 Scatter diagram comparing values for urine (ordinate) and plasma (abscissa) antipyrine half-lives from different subjects. Hatched line represents a line of equality.

Figure 2 Scatter diagram comparing values for urine (ordinate) and saliva (abscissa) antipyrine half-lives from different subjects. Hatched line represents a line of equality.
shown in Figure 1, the correlation coefficient is 0.94, with df = 31 and $P < 0.01$, which is highly significant. For the saliva-urine study, Figure 2, the correlation coefficient is 0.93, df = 21, $P < 0.01$, which is also significant.

Ten subjects had plasma and urine antipyrine half-lives determined concurrently, while on and off drug treatment. The intrasubject correlation coefficient of plasma and urinary half-lives ranged from 0.76 to 0.98 with a median value of 0.85. There were 26 studies in which plasma half-lives were longer than urine half-lives and seven studies in which plasma half-lives were shorter. Since urine half-lives tended to be somewhat shorter than plasma half-lives, we further analyzed the differences between the plasma- and urine-derived half-lives. The root-mean-square difference between the urine and plasma values compared in the same study is 1.61 h. This was made up of two components, the majority arising from the variability of these pairwise differences, which was $\sigma_{\text{diff}} = 1.426$. Additionally there is an average difference, a 'bias', which averaged 0.75 h for the 33 differences. The standard error of this bias is 0.25, so it is significantly different from zero. Thus the true average excess of plasma over urine half-life values lies between 0.25 and 1.25 h (that is, a 95% confidence interval). Note that the root-mean-square (rms) equals the square root of the sum of the bias squared and the standard deviation squared:

$$\text{rms} = \sqrt{(\text{bias})^2 + (\text{s.d.})^2}$$

Six subjects had saliva and urine antipyrine half-lives determined concurrently. The intrasubject correlation coefficient ranged from 0.74 to 0.98 with a median value of 0.75. As with plasma studies, urine half-life values tended to be shorter than saliva half-lives. The root-mean-square of the differences between saliva and urine is 1.71 h. The estimated bias is 0.192 h, which is not significantly different from zero, since its standard error is 0.33 and the standard deviation of the difference is 1.59.

Figure 3 shows the relationship between urine and plasma antipyrine half-lives in a patient with lung cancer undergoing recombinant $\beta$-interferon treatment in a phase I-II study. Antipyrine half-life increased by 150% over the course of 4 months with urine antipyrine concentration analysis reflecting the increase in half-life noted in the plasma data. The correlation coefficient for this subject was 0.97 and $P = 0.01$, which is highly significant.

Figure 4 shows the relationship between the difference in half-life estimates (obtained by subtracting the half-life determined in urine from that determined from plasma) and the coefficient of variation of the elimination rate constant estimate from urine data analysis (ordinate). Note the convergence towards the origin (V-shape).

Figure 3 Scatter diagram of urine and plasma antipyrine half-lives from one patient who received three doses of interferon. Hatched line represents a line of equality. The dose of interferon is shown near each data point in millions of units.

Figure 4 Scatter diagram of the relationship between plasma antipyrine half-life minus urinary antipyrine half-life (abscissa) and the coefficient of variation of the elimination rate constant estimate from urine data analysis (ordinate). Note the convergence towards the origin (V-shape).

The dose of interferon is shown near each data point in millions of units.

Efficient of variation of the elimination rate constant estimate from urinary data analysis obtained from the ELSFIT program (Sheiner, 1981). The figure shows a V-shaped distribution, with the smallest differences in half-life associated with the smallest coefficients of variation of elimination rate constant estimate derived from urine. Urine half-lives tend to be shorter than plasma half-lives, with the majority of the differences being positive.

Figures 1 and 2 also show that antipyrine half-lives varied over a range of 6–26 h. The lines shown are not regression lines, but lines of equality. Linear regression in this setting is not very meaningful; for example, it is possible to have very high correlation if the half-life derived from one body fluid differed consistently by several hours from that derived from another body fluid. Further, combining several points from each subject into one correlation coefficient
is not, in general, a legitimate procedure, because the data points are not independent when they come from the same subject. Treating them as independent in a combined correlation coefficient is thus invalid. In this study however, there is no evidence of an intersubject variance component, so we can legitimately compute and test a correlation coefficient.

Analysis of half-life changes in subjects on interferon or ketoconazole or no medication (data not shown) shows that the alterations observed in half-life correspond in all body fluids monitored (plasma, saliva, and urine) and is independent of drug treatment. The plasma and salivary antipyrine concentrations differ by less than 5%, as would be predicted from the small fraction of antipyrine bound to proteins in plasma. The midpoint urinary concentrations were consistently below the plasma concentration by an average of about 4.5%. The duration of the urine collection intervals in these spontaneously-voided samples ranged from 0.5 to 11 h. We therefore further analyzed these data to determine whether the length of the collection interval affected the relationship between the plasma and urine concentrations. No effect was seen, indicating that the urinary concentration is independent of both the urine flow rate and the duration of sample collection.

Discussion

We have found a useful correlation between salivary and plasma antipyrine half-lives and the half-lives determined by measuring urinary antipyrine concentrations. The close correlation between plasma and saliva half-lives is well-known and therefore saliva has been widely employed as a non-invasive sampling method for studying hepatic drug metabolism with antipyrine. The utility of urine collection as a simple, non-invasive technique for investigating hepatic metabolism is less well-established. When analysing urine data, the usual approaches are methods involving urinary excretion rate (UER) and amount remaining to be excreted (ARE), both of which are error-prone when the duration of urine collection is too short, when samples are lost or when a sample collection is missed. We have shown that the duration of urine sample collection does not affect the relationship between antipyrine concentration in urine and plasma, nor is the relationship affected by flow (Taylor & Blaschke, 1984). Thus, as long as samples are collected over two or more half-lives, an accurate estimate of antipyrine half-life can be made from urine. The method used in this study does not require a complete collection of urine samples beyond two to three half-lives. Errors in collection of a particular sample do not destroy the validity of the rest of the data. Similarly, errors in chromatographic assay for a sample do not result in a systematic or cumulative error which would drastically affect the half-life. Finally, incomplete collection or the loss of a sample does not significantly affect the data analysis.

This method, using spontaneously-voided urine antipyrine concentrations for deriving antipyrine half-life, is not without problems. In healthy normal volunteers with good urine output, the technique works excellently, but in studies with patients with varying degrees of advanced cancer (interferon studies), we found that the urine data tends to be less reliable when the urine output is less than 1.1 l day⁻¹. Urinary antipyrine concentrations in these patients with low urine output tend to show considerable scatter, with the attendant difficulty in fitting the urinary antipyrine concentration profile. Half-life estimates in this setting tend to give large confidence intervals and correlate poorly with plasma- and saliva-derived half-lives. We think this finding may be due, in part, to incomplete voiding and mixing of urine from different collection intervals in the bladder. Also there is clearly a slight discrepancy between the urine and plasma derived antipyrine half-lives. The cause of this persistent bias, which averaged 0.75 h, is unknown, but for a more accurate determination of plasma antipyrine half-life from urine data, there may be need for a 'correction factor', to correct for the bias.

This non-invasive technique of studying antipyrine metabolism could be used in pediatric patients who are unable to provide saliva specimens. Also, it may be useful in studying patients in Intensive Care Units (ICU) who frequently have bladder catheters, are unable to give saliva and in whom additional blood sampling or blood loss from such studies is undesirable.

In conclusion we have shown that plasma antipyrine half-life can be derived from antipyrine concentrations measured in spontaneously-voided urine, provided the subjects are well-hydrated and have a total urine output of 1.1 l day⁻¹ or more. This approach may be valuable in performing antipyrine clearance studies in patients unable to provide adequate numbers of plasma or saliva samples.

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References


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