Antipyrine clearance in children from single saliva samples

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The saliva clearance of antipyrine was measured in 18 children from four samples taken about 9, 13, 22 and 25 h after ingestion of 20 mg kg⁻¹. Antipyrine clearance determined from each of the samples using a volume of distribution estimated from age (A) and body weight (BW) and height (BH) \( V = 1.535 \times A + 0.339 \times BW + 0.300 \times BH - 35.63 \) \((l)\) correlated closely with clearance determined from the total elimination curve \( (r > 0.94) \). Random variation and systematic deviation were minimal when the 22 h sample was used for clearance determination \( (r = 0.98, P < 0.001) \), regression curve slope = 1.00, intercept = 0.58 and residual variance = 4.02). The one-sample method for determination of antipyrine saliva clearance is non-invasive, easy to perform and acceptable to children.

**Keywords** antipyrine clearance one-sample method children

**Introduction**

Measurement of antipyrine clearance is widely used to assess quantitative hepatic function and drug metabolizing capacity (Vesell, 1979) and is, as such, also useful in children (Forsyth et al., 1982; Moreland et al., 1982). The substitution of saliva for plasma makes the test more acceptable (Fraser et al., 1976). Recently, a simplification of the determination of antipyrine clearance using only one sample has been described in adult humans (Døssing et al., 1982) and rats (Piilsgaard & Poulsen, 1984). The present study was undertaken to investigate the applicability in children of this simple method.

**Methods**

Eighteen children participated in the study (age = 6.67 ± 1.45 years; body weight = 24.7 ± 4.28 kg; body height = 124.7 ± 8.3 cm; mean ± s.d.). Informed consent was obtained from the parents and the investigation was approved by the Ethics Committee of Copenhagen County. After an overnight fast the children ingested antipyrine 20 mg/kg dissolved in orange juice. Nine to 10 h later, before bedtime, early in the following morning and about 25 h after dosage 0.5 ml of saliva was collected without stimulation of saliva secretion. Samples were stored at −20°C until analysis by h.p.l.c. as described by Boel et al. (1984), although we used an automatic system from Perkin-Elmer fitted with a Novapak® 5 μ C 18 column.

Antipyrine clearance (CL), elimination constant \( k_e \) and apparent volume of distribution \( (V) \) were calculated from the logarithm of saliva concentration \( Vs \) vs time curve using the following equations: \( CL = k_e \times V \), \( V = D/C(o) \), where \( k_e \) is the slope of the curve, \( D \) is the ingested dose and \( C(o) \) is the extrapolated drug concentration at zero time.

Using a multiple linear regression analysis of age in years \( (A) \), body weight in kg \( (BW) \) and height in cm \( (BH) \) on the measured \( V \) a formula for an estimated \( V(\text{est}) \) was established: \( V_{\text{est}} = A \times 1.535 \times BW + 0.339 \times BH \times 0.300 - 35.63 \) \((l)\). Using this estimated \( V \) the simplified one-sample antipyrine clearance \( \text{OSAC} \) was calculated as: \( \text{OSAC} = (ln(D/V_{\text{est}}) - lnC(t)) \times V_{\text{est}}/t \), where \( C(t) \) is the saliva concentration of antipyrine at time \( t \) (Døssing et al., 1982). Linear regression analysis of \( \text{OSAC} \) on \( CL \) (four samples) was performed by the least square method for each sampling time. As shown earlier, the bias introduced in the correlation coefficient, \( r \), from interdependency of \( \text{OSAC} \)
and CL sharing a datapoint is negligible when correlation is high, i.e. if \( r \) is larger than 0.9 (Dössing et al., 1982).

Deviation of regression curve slope from 1 and intercept from 0 was evaluated statistically by means of Student's \( t \)-test. \( P \) values less than 0.05 were considered statistically significant.

**Results**

The antipyrine clearance estimated from the four samples of saliva was 30.2 ± 9.8 ml min\(^{-1}\) (mean ± s.d.). The volume of distribution \( (V) \) was 20.4 ± 6.3 l and the half-life \( (t_{1/2}) \) was 8.1 ± 2.0 h. A linear regression analysis of the estimated \( V \) on measured \( V \) gave a correlation coefficient, \( r = 0.900 \) \( (P < 0.01) \), curve slope 1.004 \( (P = 0.98) \) and intercept 0.478 \( (P = 0.85) \) and residual variance \( s^2 = 7.45 \) indicating no systematic deviation and little random variation.

Plots of one-sample antipyrine clearance (OSAC) on antipyrine clearance calculated from the four samples (CL) are shown in Figure 1 and results of the corresponding regression analysis are summarized in Table 1. At all sampling times correlations between OSAC and CL were high \( (r \) above 0.94) and no statistically significant systematic deviations were recorded. The OSAC calculated from sample 3 obtained at about 22 h after antipyrine ingestion gave the most accurate estimate of CL with virtually no systematic deviation (intercept approximately 0 and slope 1), as well as minimal random variation expressed by the low residual variance.

**Discussion**

In this study we have demonstrated that antipyrine clearance in children can be determined by a simple non-invasive method requiring only one sample of saliva. The theoretically ideal time for taking this sample for clearance determination can be calculated as

\[
t = \frac{1 + \sigma^2/\omega^2}{k_{el}},
\]

where \( k_{el} \) is the elimination constant and \( \sigma \) and \( \omega \) are the coefficients of variation of the drug concentration and the volume of distribution, respectively (Dössing et al., 1983). With common values \( t \) is between \( 1/k_{el} \) and \( 2/k_{el} \), i.e. 11 to 23 h in our material. However, if antipyrine is to be ingested in a fasting state most of this period covers the night hours. On the other hand, it is better to collect the sample too late rather than too early (Dössing et al., 1983). Our
Table 1  Results of linear regression analysis of one-sample antipyrine clearance (OSAC) on antipyrine clearance (CL) determined from the complete drug elimination curve and difference between CL and OSAC

<table>
<thead>
<tr>
<th>Sample</th>
<th>t (h) (mean ± s.d.)</th>
<th>CL-OSAC (ml min⁻¹) (mean ± s.d.)</th>
<th>r</th>
<th>b</th>
<th>a</th>
<th>s²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.61 ± 0.49</td>
<td>2.54 ± 7.71</td>
<td>0.94</td>
<td>1.11 (0.91–1.30)</td>
<td>3.88 (−10.09–+2.33)</td>
<td>16.6</td>
</tr>
<tr>
<td>2</td>
<td>12.5 ± 0.86</td>
<td>−0.47 ± 3.37</td>
<td>0.94</td>
<td>0.93 (0.77–1.10)</td>
<td>2.46 (−2.89–+7.81)</td>
<td>12.3</td>
</tr>
<tr>
<td>3</td>
<td>21.8 ± 0.66</td>
<td>−0.56 ± 1.89</td>
<td>0.98</td>
<td>1.00 (0.90–1.10)</td>
<td>0.58 (−2.48–+3.64)</td>
<td>4.0</td>
</tr>
<tr>
<td>4</td>
<td>24.9 ± 0.69</td>
<td>−0.24 ± 3.17</td>
<td>0.95</td>
<td>0.92 (0.77–1.08)</td>
<td>2.55 (−2.44–+7.54)</td>
<td>10.7</td>
</tr>
</tbody>
</table>

\[ t = \text{time after antipyrine ingestion}, \ r = \text{correlation coefficient}, \ b = \text{slope}, \ a = \text{intercept}, \ s² = \text{residual variance}; \] 95% confidence intervals in brackets

Results show that antipyrine clearance can be determined from one saliva sample collected about 22 h after drug without any systematic deviation and minimal random variation from the standard method based on four samples.

Determination of antipyrine clearance by the one-sample method is very simple and easy, particularly in children. Moreover, the use of h.p.l.c. assays requiring only 100 μl of saliva for antipyrine determination renders stimulation of saliva, e.g. with citric acid (Bacon et al., 1978), unnecessary. This makes the test even more acceptable. However, in contrast to the multiple sample approach, the one-sample method will not measure the volume of distribution and does not allow detection of single errors in sampling time or analysis. On the other hand, computer studies have shown that the one-sample method is very resistant to errors in the estimation of or changes in the volume of distribution (Pilsgaard & Poulsen, 1984).

The one sample saliva method for antipyrine clearance determination in children is convenient for longitudinal and large-scale cross sectional studies. In our experience, parents, carefully instructed at a previous hospital visit, can administer a measured dose of antipyrine and collect saliva from their children correctly. This has broadened the application of the antipyrine test, allowing for studies performed on an out-patient basis.

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References


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