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Assessment of antipyrine kinetics from saliva or plasma: influence of age

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Abstract

The purpose of this study was to investigate whether antipyrine estimation in saliva provides valid information on plasma antipyrine clearance (AP_{Cl}) and can be useful as an index of changes in drug metabolism with age. Antipyrine kinetics was studied in 93 elderly (mean age 82 years) and 23 young (mean age 29 years) volunteers. Plasma antipyrine half-life ($APt_{1/2}$) increased and plasma AP_{Cl} declined with age. No significant difference between plasma- and saliva-derived parameters was found in either young or old subjects. However, the saliva/plasma AC_{Cl} ratio tended to increase with age. A highly significant correlation between saliva and plasma AP_{Cl} or $APt_{1/2}$ was found in young subjects. Values were less closely related in the elderly and the slope of the saliva/plasma AP_{Cl} relationship was significantly different in both groups of subjects. Residual variance was higher in the regressions corresponding to the elderly. The findings in the study indicate that the relationship between saliva and plasma kinetics in young subjects becomes less reproducible with age.

Keywords: Age; Antipyrine; Oxidative metabolism; Plasma; Saliva

1. Introduction

The increasing incidence of chronic disease in the elderly, with the prescription of a large number of drugs and enhanced opportunities for drug interaction, and the decline in the function of organs critical for drug elimination, such as kidney and liver, increases the need to estimate drug metabolism and pharmacokinetics in this age group. Extensive literature references show the existence of differences in the pharmacokinetics of many drugs between young and old subjects [1].

Antipyrine has been extensively used as a model compound to study the influence of disease, drugs and environmental factors on hepatic drug-metabolizing capacity. This molecule is rapidly and completely absorbed from the gastrointestinal tract and extensively metabolized by the cytochrome P-450 liver enzymes. Its elimination is independent of protein binding and its clearance is not limited by liver blood flow, which declines with age. Thus, antipyrine clearance (AP_{Cl}) constitutes a sensitive indicator of hepatic microsomal enzyme activity that can provide specific information of hepatic function in the elderly. Studies have reported lower AP_{Cl} and long half-lives ($APt_{1/2}$) of antipyrine in subjects over 65 years compared with those in subjects under 40 years [2–6].

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Owing to its low pK_a and its small degree of plasma protein binding, antipyrine is distributed in total body water; highly reproducible significant correlations between its saliva and plasma concentrations have been reported in young subjects [7–9]. Since antipyrine plasma and saliva clearances do not appear to differ significantly [4,10] saliva concentrations have been used over recent years to characterize antipyrine elimination even in aged subjects [4,5]. However, information on the extent of the relationship between saliva and plasma AP_{Cl} and on the validity of saliva estimations in the elderly is absent. The present study was undertaken to investigate whether antipyrine estimation in saliva provides valid information on plasma AP_{Cl} and can be useful as an index of changes in drug metabolism with age.

2. Experimental

2.1. Subjects and procedures

AP_{Cl} was measured in 93 elderly subjects from a nursing home of the Inserso, a large Spanish urban institution for the care of the elderly. Twenty nine subjects were men and 69 were women. Their mean age was 82 ± 9 years; the age range was 60–100 years. A group of 23 young subjects served as control (11 males and 12 females; mean age 29 ± 5 years; age range 21–39 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. Therapeutic regimes and diets were also stable, which ensured the reproducibility of antipyrine kinetic parameters and the validity of the information obtained [5].

Antipyrine (1 g) was administered orally and plasma and saliva samples were collected after 24 h. Blood (5 ml) was drawn, with the subjects in the seated position, from the cubital vein with minimal stasis, and samples were centrifuged at 3000 rpm for 15 min to obtain plasma. The flow of saliva was stimulated by chewing a 5×5 cm patch of inert Parafilm. Both plasma and saliva samples were stored at -20 °C until analysis.

Antipyrine elimination was estimated using a single time–concentration point. This method has been demonstrated to be minimally affected by changes in the volume of distribution and avoids the systematic deviation of the method that arises from the collection of multiple samples [11].

2.2. Analytical methods

Antipyrine concentration was determined by a high-performance liquid chromatographic technique [12,13]. To 1.0 ml of plasma or saliva were added 100 µl of a solution containing 400 µg ml phenacetin in ethanol and 100 µl of 2 M NaOH. After extraction with 5 ml of dichloromethane–*n*-pentane (1:1, v/v) on a vortex for 15 s, the organic layer was collected and evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 100 µl of eluent if 25 µl was injected into the chromatograph.

The HPLC system consisted of a SP8000 pump (Spectra Physics), a Spheri-10 RP-18 10 µm column (Brownlee Columns), and a Spectra Chrom 200 detector (Spectra Physics) set at 254 nm. The column temperature was controlled at 40 °C by a water circulator. The mobile phase of 0.1 M sodium acetate–acetonitrile–TEA (92:7.5:0.5, v/v/v) (pH 6.6) was delivered at a flow-rate of 3.5 ml min^{-1}.

AP_{Cl} and $APt_{1/2}$ were calculated by the equations [12,14]

$$AP_{Cl} = \frac{\ln(D/V_d) - \ln C_t}{t} V_d$$

$$APt_{1/2} = \frac{0.693 V_d}{AP_{Cl}}$$

where D was the dose of antipyrine given, V_d was the apparent volume of distribution, t was the time of sampling and C_t was the corresponding concentration. AP_{Cl} was expressed in ml kg^{-1} min^{-1} or ml kg^{-1} and $APt_{1/2}$ was expressed in h.

V_d was calculated from a multiple regression analysis of age, body weight (BW) and body height (BH), according to the following formula [14]:

$$V_d(l) = 0.3625 \times BW(kg) + 0.2239 \times BH(cm) - 0.1387 \times age(years) - 14.47 \text{ for men}$$

$$V_d(l) = 0.2363 \times BW(kg) + 0.1962 \times BH(cm) - 0.0272 \times age(years) - 10.26 \text{ for women}$$

Table 1
Values[a] for antipyrine kinetic parameters in young and elderly subjects

	Young	Elderly
Age (years)	29 ± 5 (21–39)	82 ± 9 (60–100)[b]
V_d(l)	37.49 ± 4.95 (28.53–50.47)	29.81 ± 3.42 (21.73–45.82)[b]
V_d(l kg^{-1})	0.60 ± 0.09 (0.49–0.67)	0.55 ± 0.10 (0.41–0.76)[b]
AP_{Cl} saliva (ml min^{-1})	39.11 ± 10.93 (20.98–76.65)	21.34 ± 7.79 (4.41–50.05)[b]
AP_{Cl} saliva (ml kg^{-1} min^{-1})	0.63 ± 0.14 (0.36–1.05)	0.38 ± 0.19 (0.07–0.94)[b]
$APt_{1/2}$ saliva (h)	11.70 ± 2.30 (7.12–18.67)	19.11 ± 10.73 (6.76–101.14)[b]
AP_{Cl} plasma (ml min^{-1})	38.91 ± 12.42 (22.08–78.05)	20.70 ± 7.79 (4.54–44.72)[b]
AP_{Cl} plasma (ml kg^{-1} min^{-1})	0.63 ± 0.13 (0.38–1.07)	0.37 ± 0.19 (0.08–0.88)[b]
$APt_{1/2}$ plasma (h)	11.93 ± 2.56 (6.83–18.49)	19.56 ± 8.83 (6.64–75.76)[b]

[a] Values are: mean ± S.D. (range); V_d, apparent volume of distribution; AP_{Cl}, antipyrine clearance; $APt_{1/2}$, antipyrine half-life.
[b] = $p < 0.05$ compared with young subjects.

2.3. Statistical methods

The data were expressed as mean ± sd. 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 analysis was performed with the method of Pearson. Slopes of the regression lines were compared by the method of Theil. Analysis were run on the SPSS for Windows statistical package, version 5.0.2. (SPSS Federal Systems, Chicago, IL).

3. Results

Table 1 shows kinetic data derived from plasma and saliva samples in both young and old subjects. Plasma $APt_{1/2}$ increased and plasma AP_{Cl} declined with age whether or not this variable was corrected for weight. The apparent volume of distribution was lower in the elderly with or without correction for weight.

No significant difference between plama- and saliva-derived parameters was found in either young or old subjects. However, saliva AP_{Cl} in the elderly was 3.1% higher and saliva $APt_{1/2}$ 2.4% lower than the corresponding plasma values, with smaller differences in young subjects (+0.5% and −2.0%, respectively) (Table 1). The saliva/plasma AP_{Cl} ratio tended to increase with age (1.03 ± 0.09 versus 1.00 ± 0.05 in young subjects).

Plots of saliva AP_{Cl} and saliva $APt_{1/2}$ against plasma AP_{Cl} and plasma $APt_{1/2}$ together with the corresponding regression analysis of the results are shown in Fig. 1. A highly significant correlation between saliva and plasma AP_{Cl} was found in young subjects. Values were less closely related in the elderly and the slope of the relationship was significantly different in both groups of subjects. The relationship between saliva and plasma $APt_{1/2}$ of antipyrine was closer in young than in old subjects. Antipyrine $APt_{1/2}$ values were less closely related than AP_{Cl} values in both young and old subjects. Residual variance, an expression of random variation between two estimates, was higher in the regressions corresponding to the elderly.

4. Discussion

Impairment of antipyrine metabolism in elderly subjects was first reported by O'Malley et al. [15], who found significant increases in mean $APt_{1/2}$ in a group of 18 geriatric patients. This was later confirmed by different authors [2–6]. The present data are consistent with previously reported values and confirm that aging is accompanied by a decline in the apparent volume of distribution and clearance of antipyrine, and by a significant increase in antipyrine half-life.

Vesell et al. [8] and Welch et al. [16] were the first to demonstrate that both plama and saliva concentrations could be used to characterize antipyrine pharmacokinetics. Vessel et al. [8] found that the apparent volume of distribution of antipyrine was similar in plasma and saliva when the drug was administered orally. Welch et al. [16] reported that in rat and man the distribution ratio of antipyrine between saliva and plasma was essentially unity after an oral or parenteral dose. They also demonstrated that the antipyrine saliva concentration is not a

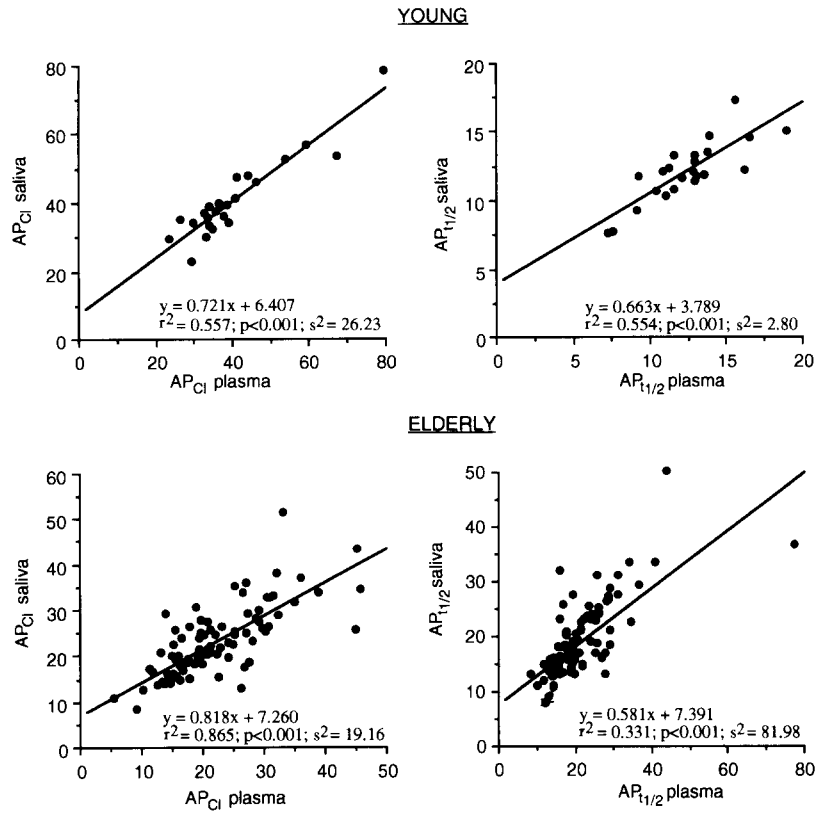

Fig. 1. Correlation between saliva and plasma antipyrine clearance (AP_{Cl}) and antipyrine half-life ($APt_{1/2}$) in young and elderly subjects. r^2, regression coefficient; s^2, residual variance.

function of the salivary flow-rate, since increases in salivation caused by pilocarpine did not change its plasma/saliva concentration ratio [16]. Thereafter, a number of authors have used the elimination rate of antipyrine from saliva to estimate the influence of different environmental factors, drugs and clinical states on antipyrine metabolism [9,17–19].

Antipyrine saliva sampling is easier to perform and much more acceptable than venepuncture to volunteers, especially in the elderly. For this reason, different authors have used antipyrine elimination from saliva as an index of changes in drug metabolism with age [4,5]. This method, however, would only be valuable if it could reflect intravenous antipyrine clearance. The present work has shown in young subjects an AP_{Cl} saliva/plasma ratio of 1.00 and a correlation coefficient of 0.93. These results are similar to those reported by Fraser et al. [10] in a group of 32-year-old people, with a correlation coefficient of 0.96, and by Danhof et al. [20], with a correlation coefficient of 0.91. Results for the elderly, however, are notably different. First, there is a tendency for the AP_{Cl} saliva/plasma ratio to increase with age; there is also a decrease in the correlation coefficient with age together with an increase in the residual variance for both AP_{Cl} and $APt_{1/2}$ regression lines. These data are expressions of higher dispersion and random variation in data corresponding to the eldery.

Different mechanisms can account for the difference between saliva and plasma antipyrine kinetics in the elderly. The slightly higher increase in saliva clearance than in plasma clearance in the elderly could reflect some presystemic mucosal metabolism or altered estimates of volume of distribution [21]. The possibility also exists that saliva sampling after oral administration could be complicated by its residual presence in the oral cavity. However, these problems are most probably of minor relevance, because antipyrine clearance was determined in the present study using a single time–concentration point [13]. Computer studies have shown that the one-sample method is very resistant to errors in the estimation or in changes in the volume of distribution [22]. Additionally, the saliva sample is taken 24 h after antipyrine administration, which guarantees the absence of the drug from the oral cavity.

In summary, the findings in this study indicate that significant correlations between saliva and plasma kinetics on young subjects become less reproducible with age. Although the exact mechanisms involved still require further clarification, caution must be adopted when saliva concentrations are used to characterize antipyrine pharmacokinetics in the elderly.

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