The Effect of Age and Sex on Metabolism and Urinary Excretion of Antipyrine

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Background. Drug-metabolizing capacity is generally reduced in the elderly. The purpose of this investigation was to study antipyrine clearance and metabolite excretion in old subjects of both sexes.

Methods. Saliva clearance of antipyrine and the production clearances of antipyrine metabolites were studied in young and elderly volunteers of both sexes. Seventy-six elderly subjects (mean age 81 years) were compared with a group of 24 young subjects (mean age 29 years).

Results. After oral administration, salivary antipyrine clearance declined with age in both males and females, whether or not this variable was corrected for weight, and antipyrine half-life was significantly prolonged in elderly groups of either sex. The percentage urinary excretion of the antipyrine metabolites (hydroxymethylantipyrine, HMA; norantipyrine, NORA; and 4-hydroxyantipyrine, OHA) was reduced at 48 h in the elderly compared to young subjects by 23%, 31%, and 10%, respectively, in males, and by 41%, 41%, and 24%, respectively, in females. The formation clearance of HMA was reduced by 47% in males and by 52% in females. NORA clearance declined by 42 and 56%, respectively, in males and females. A decrease of 30% in males and 44% in females was observed in OHA clearance.

Conclusions. The findings suggest that aging leads to altered disposition of antipyrine in both males and females and that the main metabolic pathways of the compound are not different in the elderly.

Antipyrine is a compound with a low hepatic extraction ratio and minimal protein binding that has been used in the study of hepatic drug oxidation in humans. This drug has excellent absorption and is extensively metabolized by the cytochrome P-450 liver enzymes. Antipyrine clearance is not limited by liver blood flow, which declines with age, and constitutes a sensitive marker of hepatic microsomal enzyme activity in the elderly (1).

Antipyrine is metabolized in humans to three major metabolites, 3-hydroxymethylantipyrine (HMA), 4-hydroxyantipyrine (OHA), and norantipyrine (NORA). The formation of each of the three metabolites is dependent on one or more selective forms of cytochrome P-450, and characterization of the main individual antipyrine metabolite disposition may provide a more specific information about metabolic pathways activity than total plasma or saliva clearance alone.

Lower clearance and longer half-lives of antipyrine have been reported in elderly subjects in a number of studies (2–6). Differences in enzyme inducibility attributed to smoking, alcohol, and other drugs have been proposed to account for these changes (2,4), and variables that can predict a low metabolizing capacity, including factors other than age, have been identified (6). It has been postulated that the formation of the individual antipyrine metabolites is influenced by aging in different ways, with a greater suppression for norantipyrine suggesting a selective inhibition of various cytochrome P-450 isoenzymes (7).

Different investigations have revealed a sex difference in antipyrine disposition, with significantly lower antipyrine clearances in young women than in men (8,9). Although it has been proposed that the decline in antipyrine clearance with age is sex-linked (3), data are conflicting since higher decrements in men (3) or in women (10), as well as no difference (6), have been described. In any case, differences in rate decrease of antipyrine metabolite formation between men and women have not been studied up to the present.

The purpose of our study was to assess the influence of age on the different metabolic pathways of antipyrine and to determine whether the activities of individual cytochrome P-450 isoenzymes are differentially affected in male and female elderly subjects. Saliva clearance of antipyrine and the production clearances of antipyrine metabolites have been compared in young and elderly volunteers of both sexes.

METHODS

Compounds
Antipyrine (Sigma, St Louis, MO), NORA (Aldrich, Milwaukee, WI), OHA (Aldrich, Milwaukee, WI), and glusulase (Sigma, St Louis, MO) were obtained from commercial sources in the highest purity available. HMA was generously donated by Dr. S. Loft (Institute of Pharmacology, Copenhagen, Denmark).

Subjects and Procedures
Antipyrine metabolism was studied in 76 elderly subjects who were residents of a nursing home of the Inserso,
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a large Spanish urban institution for the care of the elderly. Twenty-seven subjects were men, and 49 were women. Their mean age was 81 years; age range was from 60 to 100 years. A group of 24 young subjects served as controls (10 males and 14 females, mean age 29 years, age range 21–39 years). The study was approved by the Ethics Committee of the University of Leόn. Subjects gave informed consent before entering the study. Inclusion required that they be medically stable, suffering no hepatic or renal disease, and with no hospitalizations within 1 month before the study. All subjects were nonsmokers.

Antipyrine (1 g) was administered orally following a 10-h fast. Saliva samples were collected at 24 h (11). Urine was collected at 12, 24, 36, and 48 h after antipyrine ingestion, in containers with an antioxidant (sodium metabisulfite). The urine volume was measured, and aliquots from each interval collection were frozen at -40 °C until assay.

Analytical Methods

Antipyrine concentration in saliva was determined by a high-performance liquid chromatography (HPLC) technique (12) as follows: to 1.0 mL of saliva, 100 uL of a solution containing 400 µg phenacetin/mL ethanol and 100 µL 2 N NaOH were added. After extraction with 5 mL of dichloromethane-n-pentane (1:1, v/v) on a whirlmixer 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, of which 25 µL were injected into the HPLC system.

Antipyrine and its three main metabolites were measured in urine (13) as follows: to 1.0 mL of urine, 50 mg of sodium metabisulfite, 1 mL of 1.0 M acetate buffer pH 5.0 and 50 µL of glusulase were added. Samples were incubated in a shaker bath at 37 °C for 3 h. Then to each tube, 0.5 mL of the buffer and 20 µg of phenacetin were added and the samples were subsequently extracted with 5 mL of ethyl acetate by shaking them on a horizontal mixer for 10 min at 90 cycles/min. After centrifugation at 400g for 10 min, the organic layer was collected and evaporated to dryness. The residue was dissolved in 100 µL of eluent, of which 25 µL were injected into the HPLC system.

The HPLC system consisted of an SP8000 pump (Spectra Physics, San José, CA), a Spheri-10 RP-18 10-µm column (Brownlee Columns, San José, CA), 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% triethylamine, pH = 6.6, was delivered at a flow rate of 3.5 mL/min.

Linear calibration with correlation coefficients better than 0.990 was obtained for all assay procedures. Limit of quantitation for antipyrine in saliva was 0.1 µg/mL. Limits of quantitation in urine were 2 µg/mL for antipyrine, 3-hydroxyxymethylantipyrine, and norantipyrine, and 5 µg/mL for 4-hydroxyantipyrine.

Pharmacokinetic Analysis

Salivary antipyrine clearance (ClAp) and half-life (t1/2) were calculated by the equations (11):

\[ Cl_{AP} = \frac{\ln(D/Vd) - \ln C_t}{t} \times Vd \]

\[ t_{1/2} = \frac{0.693 \times Vd}{Cl_{AP}} \]

where D is the dose of antipyrine given, Vt is the apparent volume of distribution, t is the time of sampling, and C, is the corresponding concentration. Vt was estimated from a multiple regression analysis of age, body weight, and height (11). The calculation of antipyrine pharmacokinetic parameters using this simplified approach based on estimated distribution volumes has been validated in young adults (11) and children (14) and more recently in the elderly (6).

The renal clearance of antipyrine and the formation clearances of antipyrine metabolites (Clm) were calculated according to (15,16)

\[ Cl_m = f_m \times Cl_{AP} \]

where fm is the fraction of the dose excreted as antipyrine or as a particular metabolite (m).

Statistical Methods

The data were expressed as means ± SEM. Statistical analysis was performed by analysis of variance. When the analysis indicated the presence of significant differences, means were compared by the Newman-Keuls test (17). Metabolism measures separated by sex were also compared by analysis of covariance with one factor (sex) and one covariate (age) (17), with p values less than .05 considered significant. Analyses were run on the STATISTICA statistical package (Statsoft, Tulsa, OK).

RESULTS

Antipyrine in Saliva

Table 1 summarizes the salivary pharmacokinetic parameters in both young and elderly subjects. Mean antipyrine clearance declined with age in both males or females, whether or not this variable was corrected for weight (males, -39 and -44% with and without normalization for body weight, respectively; -46 and -43% for the same parameters in females). Antipyrine half-life was significantly prolonged in elderly groups of either sex (males, +54%; females, +68%). Mean volume of distribution was smaller with or without correction for body weight (males, -10 and -19%, respectively; females, -16 and -11%, respectively). Antipyrine clearance without correction for body weight was significantly lower in women than in men (–23% in young subjects and –21% in the elderly), but there was no significant difference when values were corrected for weight. No significant difference in antipyrine half-life was observed between men and women in the young or elderly groups.

Antipyrine and Metabolites in Urine

The urinary excretion of the antipyrine metabolites, expressed as percentage of the dose, is shown in Figure 1. Mean percentage excretion of each of the three metabolites was significantly reduced with age in both males or fe-
Table 1. Values for Saliva Antipyrine Kinetics in Young and Elderly Subjects of Both Sexes

<table>
<thead>
<tr>
<th></th>
<th>Young Males (n = 27)</th>
<th>Young Females (n = 49)</th>
<th>Elderly Males (n = 10)</th>
<th>Elderly Females (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30 ± 1</td>
<td>28 ± 2</td>
<td>80 ± 1*</td>
<td>81 ± 1*</td>
</tr>
<tr>
<td>Vd (L)</td>
<td>42.3 ± 1.6</td>
<td>33.9 ± 0.6*</td>
<td>34.4 ± 1.1*</td>
<td>30.3 ± 0.4*</td>
</tr>
<tr>
<td>Vd (L/kg)</td>
<td>0.58 ± 0.02</td>
<td>0.61 ± 0.01</td>
<td>0.52 ± 0.02*</td>
<td>0.51 ± 0.01*</td>
</tr>
<tr>
<td>Clm (mL/min)</td>
<td>44.9 ± 4.2</td>
<td>34.7 ± 2.5#</td>
<td>25.3 ± 2.2*</td>
<td>19.9 ± 1.0#*</td>
</tr>
<tr>
<td>Clm (mL/min/kg)</td>
<td>0.62 ± 0.06</td>
<td>0.62 ± 0.04</td>
<td>0.38 ± 0.03*</td>
<td>0.34 ± 0.02*</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>11.3 ± 0.6</td>
<td>11.8 ± 0.8</td>
<td>17.4 ± 1.4*</td>
<td>19.8 ± 1.2*</td>
</tr>
</tbody>
</table>

Note: Data are mean values ± SEM. Vd, estimated apparent volume of distribution; Clm, apparent antipyrine clearance; t1/2, antipyrine half-life.
#Significantly different from males (p < .05).
*Significantly different from young, same sex (p < .05).

Figure 1. Recovery of antipyrine and its metabolites in urine in young and elderly subjects of both sexes. Values are expressed as percentage of the dose. HMA, 3-hydroxymethylantipyrine; NORA, norantipyrine; OHA, 4-hydroxyantipyrine. #Significantly different from males (p < .05). *Significantly different from young, same sex (p < .05).

males. HMA, NORA, and OHA excretion in 48 h was reduced by 23, 31, and 10%, respectively, in males, and by 41, 41, and 24%, respectively, in females. The percentage excretion of unmetabolized antipyrine did not change significantly with age. The total percentage of the dose excreted in 48-h urine as unmetabolized antipyrine plus the three main metabolites was significantly reduced in elderly groups of either sex (for males, 43.7 ± 2.7% vs 51.5 ± 4.1% in young; p < .05; for females, 38.2 ± 2.0% vs 54.3 ± 4.4% in young, p < 0.5). Urinary excretion of each metabolite did not differ between young males and females. Values for HMA and OHA excretion were significantly less in elderly females compared with elderly males at 48 h (~17%).

The renal clearance of antipyrine and the formation clearance of its metabolites in 48 h are summarized in Table 2. There was no significant change with age in the
renal clearance of antipyrine, although values were reduced by 18% in females. Significant declines in HMA, NORA, and OHA formation clearances were observed in both sexes. The formation clearance of HMA was reduced by 47% in males and by 52% in females. NORA clearance declined by 42 and 56%, respectively, in males or females. A decrease of 30% in males and 44% in females was observed for OHA clearance. Formation clearances for each of the three metabolites did not differ significantly between young males and females, but were smaller in elderly females than in elderly males (HMA -20%; NORA -22%; OHA -20%).

Figure 2 shows plots of formation clearances of the different antipyrine metabolites versus age, separated by sex. Analysis of covariance indicated the existence of a significant difference (p < .05) between males and females only for OHA clearance.

**DISCUSSION**

Age-related changes in drug metabolizing capacity are of potential clinical importance because of the higher incidence and increased risk of drug toxicity or drug interactions in the elderly. Literature documenting the decline of antipyrine metabolism with age in man is expanding, as shown in Table 3. O'Malley et al. (8) were the first to report that antipyrine metabolism is impaired in elderly subjects, describing significant increases of mean antipyrine half-life in a group of 18 geriatric patients. This observation has been subsequently confirmed by other authors. Thus, Liddell et al. (18), Swift et al. (19), and Vesell et al. (5) have found longer elimination half-lives in the elderly, and Bach et al. (20) reported a reduction with age in both antipyrine clearance and volume of distribution. Some results are nevertheless conflicting. Greenblatt et al. (3) reported reduced antipyrine clearance with age in both sexes, but significant prolonged half-lives were found only in males. Vestal and Wood (2) and Posner et al. (7) observed closely similar antipyrine half-lives in elderly subjects and young adults, despite the reduction in its systemic clearance in the elderly. No change with age in antipyrine half-life has been reported in rats (21). The variable nature of these results may be due to different factors, including the number of subjects studied or the separation of groups by sex. Our data are consistent with previous reports (6) and confirm that aging is accompanied by a decline in the apparent volume of distribution and a greater decline in clearance of antipyrine as well as a significant increase in antipyrine half-life in both men and women.

Drug-metabolizing capacity may decrease in the elderly as a primary abnormality, but the possibility also exists that reduced antipyrine clearance is not attributable directly to aging but rather to age-related factors that could affect drug metabolism. Different variables that can predict a low metabolism with age have been recently identified (6). Although suggestions have been made that age may be associated with impairment of the rate and extent of drug absorption from the gastrointestinal tract, the rate of antipyrine absorption appears to be, if anything, more rapid in the elderly than in young volunteers of both sexes (22). Association of increased antipyrine clearance with smoking in young adults that is not observed in the elderly has been

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**Table 2. Apparent Antipyrine Renal and Metabolite Formation Clearances (in mL/min/kg) in Young and Elderly Subjects of Both Sexes**

<table>
<thead>
<tr>
<th></th>
<th>Young Males</th>
<th>Young Females</th>
<th>Elderly Males</th>
<th>Elderly Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMA</td>
<td>0.093 ± 0.017</td>
<td>0.081 ± 0.009</td>
<td>0.049 ± 0.005*</td>
<td>0.039 ± 0.004*</td>
</tr>
<tr>
<td>NORA</td>
<td>0.156 ± 0.018</td>
<td>0.163 ± 0.012</td>
<td>0.091 ± 0.010*</td>
<td>0.071 ± 0.006*</td>
</tr>
<tr>
<td>OHA</td>
<td>0.324 ± 0.003</td>
<td>0.318 ± 0.023</td>
<td>0.226 ± 0.021*</td>
<td>0.179 ± 0.008*</td>
</tr>
<tr>
<td>Clrenal</td>
<td>0.093 ± 0.005</td>
<td>0.049 ± 0.007</td>
<td>0.037 ± 0.005</td>
<td>0.038 ± 0.003</td>
</tr>
</tbody>
</table>

Note: Data are mean values ± SEM. HMA, 3-hydroxymethylantipyrine; NORA, norantipyrine; OHA, 4-hydroxyantipyrine; Clrenal, renal clearance of antipyrine.

*Significantly different from males (p < .05).

†Significantly different from young, same sex (p < .05).
linked to impaired ability to respond to enzyme induction with age (2). A similar increase in antipyrine clearance among smokers in both young and old subjects, however, was previously reported by us (6).

Although a reduction in antipyrine clearance can provide general information regarding altered drug metabolism, the cytochrome P-450-dependent drug metabolic system consists of many isoenzymes of partly overlapping substrate specificities, some of which contribute to the biotransformation of antipyrine. Additional information about the effects of age on individual metabolic processes may be gained by characterization of the production and urinary excretion of the three main antipyrine metabolites, HMA, NORA, and OHA. These metabolites are produced by way of cytochrome P-450-mediated hydroxylation and N-demethylation, with subsequent conjugation and renal excretion (16). It has been previously reported that some degree of selectivity may exist in the impairment of oxidative metabolism with aging. Posner et al. (7) observed that HMA and OHA formation clearances are not different between young and old subjects, whereas NORA clearance is significantly lower in the elderly. These results could indicate that demethylation is more readily affected than hydroxylations in the elderly, in a similar way to findings in patients with alcoholic cirrhosis (23) and chronic renal failure (24). However, it should be noted that in this study an absence of significant differences between young and old subjects in antipyrine half-life and in the total recovery of antipyrine in urine was observed. Additionally, data from both men and women were pooled together. Preferential changes in the partial clearance of NORA with age have been reported in rats (21), but it is difficult to determine whether pharmacokinetic data obtained in old rats can be translated directly to the elderly.

In the present work the total percentage of administered antipyrine excreted in 48-h urine as unmetabolized parent compound plus the three main metabolites was significantly reduced in elderly subjects of either sex. The urinary excretion and the formation clearance of both HMA and NORA decreased in a slightly greater extent than those of OHA, but no clear pattern of different clearance was observed for any metabolite. This suggests the possibility of a nonselective inhibition of the different antipyrine metabolic pathways in the elderly. However, the correct interpretation of data concerning the disposition of antipyrine and its major metabolites is not easy, since the specific cytochrome P-450 isoenzymes involved in antipyrine metabolism have not been clearly identified. At least four different types of isoenzymes have been found by application of monoclonal antibodies, and the use of both inducers and inhibitors of oxidative metabolism suggests the involvement of three major gene families, CYP1, CYP2, and CYP3 (25). Recently, the involvement of CYP1A2, CYP2B6, CYP2C8/9, and CYP3A4 in antipyrine biotransformation has been observed using human liver microsomes, inhibitory drugs, and antibodies (26).

The existence of a sex difference in antipyrine disposition has been described, with young women having significantly lower antipyrine half-lives than men (3,8,9). In this work, antipyrine clearance uncorrected for body weight was significantly lower in women than in men. When corrected for body weight, clearance values were not significantly different in young and elderly subjects, just as no difference was found between the sexes in antipyrine half-life. This suggests that reduced antipyrine clearance in women could be due to a smaller liver volume (27) but not to a reduced capacity of microsomes per unit of liver.

Some studies have reported a different age-related decline in antipyrine oxidation in men and women, but results are inconclusive at present. Greenblatt et al. (3) found a more striking decrease in antipyrine clearance in males than in females, with a significant prolongation of the half-life observed only in males. Mucklow and Fraser (12), on the contrary, observed that antipyrine clearance did not change with advancing age in males, whereas it decreased in females. Data presented here indicate that antipyrine clearance decreases with age both in men and women. This is further confirmed by the reduction in urinary excretion and formation clearance of the major antipyrine metabolites in both sexes. The magnitude of the decline, as shown by analysis of covariance, is larger for OHA in women. If confirmed, the mechanisms responsible for this difference would require further clarification.

In summary, the findings in our study indicate that aging leads to the alteration of the disposition of antipyrine in both males and females, and that the main metabolic pathways of the compound are not different in the elderly. Considering the increased consumption of drugs in the elderly, often under multiple treatments, the reduced biotransformation of compounds requiring liver oxidative metabolism supposes an increased risk of side effects and drug interactions of which clinicians must be aware.

### Table 3. Age-based Studies of Antipyrine Kinetics

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>C1AP (mL/min) Young</th>
<th>C1AP (mL/h/kg) Young</th>
<th>C1AP (mL/min) Elderly</th>
<th>C1AP (mL/h/kg) Elderly</th>
<th>t1/2 (h) Young</th>
<th>t1/2 (h) Elderly</th>
</tr>
</thead>
<tbody>
<tr>
<td>O'Malley (8)</td>
<td>1971</td>
<td>34.6</td>
<td>120</td>
<td>28.2</td>
<td>17.4</td>
<td>12.7</td>
<td>14.8</td>
</tr>
<tr>
<td>Vestal (2)</td>
<td>1975</td>
<td>48.7</td>
<td>12.7</td>
<td>29.1</td>
<td>13.0</td>
<td>7.7</td>
<td>12.6</td>
</tr>
<tr>
<td>Vestal (2)</td>
<td>1979</td>
<td>29.6</td>
<td>7.7</td>
<td>43.5</td>
<td>16.4</td>
<td>8.9</td>
<td>16.4</td>
</tr>
<tr>
<td>Bach (20)</td>
<td>1981</td>
<td>33.2</td>
<td>11.5</td>
<td>50.0</td>
<td>13.7</td>
<td>11.6</td>
<td>13.6</td>
</tr>
<tr>
<td>Greenblatt (3)</td>
<td>1982</td>
<td>13.6</td>
<td>11.6</td>
<td>47.3</td>
<td>13.6</td>
<td>11.6</td>
<td>13.6</td>
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<tr>
<td>Posner (7)</td>
<td>1987</td>
<td>29.7</td>
<td>13.6</td>
<td>33.0</td>
<td>25.8</td>
<td>11.6</td>
<td>13.6</td>
</tr>
<tr>
<td>Siegmund (28)</td>
<td>1991</td>
<td>28.2</td>
<td>13.6</td>
<td>25.8</td>
<td>13.6</td>
<td>11.6</td>
<td>13.6</td>
</tr>
</tbody>
</table>

Note: C1AP, apparent antipyrine clearance; t1/2, antipyrine half-life.
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