Pharmacokinetics of Phenacetin in Phenacetin Abusers

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Although there are some reports which state that phenacetin is unlikely to be the primary causative agent in analgesic nephropathy (Prescott, 1982), 80% of the 285 phenacetin abusers observed in our clinic between 1962 and 1979 were suffering from chronic interstitial nephritis (Nitzsche et al., 1980). In order to ascertain whether phenacetin itself or one of its metabolites is responsible for this nephritis we have compared the pharmacokinetics and metabolic profile of phenacetin in 2 groups of phenacetin abusers with and without nephritis.

Phenacetin (900 mg), contained in gelatin capsules, was administered orally after an overnight fast. Blood and urine samples were collected. Plasma and urine concentrations of unchanged phenacetin and its major metabolites were determined by HPLC with spectrophotometric detection (254 nm).

Urinary elimination of phenacetin and metabolites was quantified as the total amount (percentage of dose) in 24 h.

The results suggest that the cause of nephritis in susceptible phenacetin abusers is not associated with altered elimination of either phenacetin itself or the metabolites measured in this study.

Key words: phenacetin, abuser, nephritis, metabolite, glucuronide

Introduction

During the last 20 years there have been numerous reports relating renal papillary necrosis and chronic interstitial nephritis due to the abuse of analgesic mixtures which usually contain phenacetin.

Although there are some reports which state that phenacetin is unlikely to be the primary causative agent in analgesic nephropathy, 285 phenacetin abusers were observed in our clinic between 1962 and 1979, and about 80% of these patients were suffering from chronic interstitial nephritis. However, the remaining 20%, although they had taken almost the same amount of phenacetin, had no nephritis. The metabolism of phenacetin is known to be, at least partially, polymorphic. Certain individuals ('poor metabolizers') have an impaired ability to deethylate phenacetin to its major metabolite, acetaminophen. In order to ascertain whether altered or impaired phenacetin metabolism was responsible for the nephritis we have compared the pharmacokinetics and metabolic profile of phenacetin in 2 groups of phenacetin abusers, with and without nephritis.
Tab. 1 Characteristics of Patients and Volunteers in the Two Groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age</th>
<th>Gender</th>
<th>Body weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>A</td>
<td>45.7±5.8</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>(36-54)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>48.4±7.9</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>(36-58)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Materials and Methods

Biological material

The following 2 groups of volunteers participated in this study. All subjects were informed about the aims of the study and gave their written consent to it.

Group A. Phenacetin abusers with chronic interstitial nephritis (n=7, n=number of volunteers).

Group B. Phenacetin abusers without nephritis (n=7).

Table 1 shows the individual characteristics outline of these subjects.

As is shown there the number of female volunteer is larger than that of male, this comes from the fact that about 70% of phenacetin abuser is female, but the metabolism of phenacetin in male and female is very similar.

Phenacetin (900 mg), in gelatin capsules, was given to each volunteer after an overnight fast.

Blood samples (10 ml) were withdrawn through an indwelling cannula in the antebrachial vein at the following times after drug administration: 0, 15, 30, 45 min and 1, 2, 3, 4, 5, 6, 8 and 24 hours.

Urine samples were collected during the following time intervals: 0-1, 1-2, 2-4, 4-6, 6-8, 8-12 and 12-24 hours. Urine was also collected before drug administration for the zero time blank sample. All volunteers were in hospital one night and remained mostly in bed until the final samples were collected.

Preparation of urine and plasma samples

Urine was diluted 20-fold with distilled water containing internal standard (4-fluorophenol, 400 µg/ml) and then directly analysed by HPLC. perchloric acid (0.5 ml of a 5% v/v solution in distilled water) containing internal standard (30 µg/ml) was added to 0.5 ml of plasma to precipitate proteins. The samples were then mixed with a vortex mixer, centrifuged for 20 min at 2,500 G, and the supernatant was analysed by HPLC.

High-performance liquid chromatographic (HPLC) procedure

Phenacetin and its metabolites were analysed by the HPLC method previously described. Briefly, three solvents were prepared: Solvent A = methanol, solvent B = 1% glacial acetic acid, solvent C = 0.1 M KH2PO4. Solvent B and C were used as a mobile phase (3 : 97) for the separation of the phenacetin metabolites, and solvent A, B and C were used (33 : 3 : 64) for the measurement of unchanged phenacetin.

Typically the HPLC system was operated at a flow rate of 1.2 ml/min at 3,500 p.s.i. at 45°C.

Pharmacokinetic analysis

The pharmacokinetic parameters for each individual were calculated using a multi-exponential computer programme (HP-9845). For subjects whose plasma concentration-time data were best described by a one compartment open model, according to the following equation:

\[ C = C_0(\exp(\alpha t) - \exp(\beta t)) \]

(where \( C_0 \) is apparent zero time concentration, \( \alpha \) is absorption rate constant and \( \beta \) is elimination rate constant), the following pharmacokinetic values were estimated: the time (\( T_{max} \)) to reach peak concentration (\( C_{max} \)) and the half-life for elimination (\( T_{1/2} \)). The area under plasma concentration (\( \text{AUC}_a \)) for the experimental period was calculated according to the blood level equation or trapezoidal rule. \( \text{REST AUC} \) (the AUC from the last concentration (\( C_l \)) to infinite time) was calculated from \( C_l/\beta \). \( \text{AUC}_o^\infty \) was estimated as \( \text{AUC}_o^\infty = \text{AUC}_o^\infty + \text{REST AUC} \). The apparent volume of distribution (\( V.D. \)) and systemic clearance (\( \text{Cl} \)) were calculated using the following formulae:

\[ V.D. = \frac{f \times \text{Dose}}{C_0}, \quad \text{Cl} = \frac{f \times \text{Dose}}{\text{AUC}_o^\infty} \]

where \( f \) is the fraction of the dose reaching the systemic circulation. As this parameter could not be calculated in the present study it was assumed for purpose of comparison that \( f \) was unity.
Statistical analysis

The mean and standard deviation of each parameter were tested for homoscedasticity and when variances were found to be homogeneously distributed values were compared using Student’s t-test; otherwise Wilcoxon two sample test was performed. One-way analysis of variance for repeated measurements was also employed.

Results

Application to biological materials

Urinary elimination of phenacetin and its metabolites are quantified as the total amount (percentage of dose) excreted in 24 h, which are shown in Tab. 2.

As shown in Tab. 2 the values for excretion of unchanged phenacetin and its metabolites in the group of abusers with nephritis were not significantly different from those obtained in the group of abusers without nephritis.

Plasma concentration-time curves of unchanged phenacetin were best described by a one compartment open model. In Tab. 3, AUC, T1/2, Cl, Cmax and Tmax are shown. Figure 1 shows the plasma concentration-time curves of phenacetin in two groups of phenacetin abusers with and without nephritis. Eight hours after drug administration unchanged phenacetin was no longer detectable.

<table>
<thead>
<tr>
<th>Group</th>
<th>P (%)</th>
<th>AC (%)</th>
<th>G (%)</th>
<th>S (%)</th>
<th>CY (%)</th>
<th>M (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.22±0.11</td>
<td>2.4±0.7</td>
<td>59.1±13.6</td>
<td>24.7±9.4</td>
<td>7.6±3.0</td>
<td>4.5±1.4</td>
</tr>
<tr>
<td>B</td>
<td>0.21±0.02</td>
<td>2.5±1.5</td>
<td>53.6±15.8</td>
<td>27.5±12.6</td>
<td>7.5±2.1</td>
<td>4.8±3.3</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Group</th>
<th>Cmax (μg/ml)</th>
<th>Tmax (hr)</th>
<th>T1/2 (hr)</th>
<th>AUC (μg·hr/ml)</th>
<th>Cl (ml/hr/kg)</th>
<th>V.D. (l/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.84±1.50</td>
<td>1.00±0.19</td>
<td>1.10±0.60</td>
<td>1.84±0.82</td>
<td>12.97±2.54</td>
<td>16.7±5.2</td>
</tr>
<tr>
<td>B</td>
<td>1.48±0.74</td>
<td>1.09±0.69</td>
<td>0.98±0.43</td>
<td>1.58±0.74</td>
<td>14.49±2.54</td>
<td>17.2±8.6</td>
</tr>
</tbody>
</table>

Values given are mean±S.D. There were no significant differences between groups in each parameter at the 5% level of significance.

Fig. 1 Concentration-versus-time curve of unchanged phenacetin. ○: abuser with nephritis. ▲: abuser without nephritis. mean±S.D.
Tab. 4 AUC of Phenacetin Metabolites.

<table>
<thead>
<tr>
<th>Group</th>
<th>G (µg·h/ml)</th>
<th>S (µg·h/ml)</th>
<th>P (µg·h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>173.7±80.7</td>
<td>87.7±60.3</td>
<td>52.4±25.8</td>
</tr>
<tr>
<td>B</td>
<td>163.6±56.0</td>
<td>83.0±46.2</td>
<td>51.0±10.5</td>
</tr>
</tbody>
</table>

As the plasma concentration-time curves of phenacetin metabolites could not be fitted to a simple pharmacokinetic model, AUCₐ₀-2₄ of each metabolite was calculated according to the trapezoidal rule. The results are shown in Tab. 4.

There were no significant differences between groups A and B in each of the parameters. In 2 subjects in group A (patients with nephritis) the sulphate conjugate could not be determined because of endogenous components in the plasma which interfered with the assay.

Discussion

Phenacetin is a mild analgesic agent, the majority of which undergoes dealkylation to form acetaminophen; this reaction being catalysed by a microsomal, cytochrome P-450 enzyme system. One or more of the cytochrome P-450 isozymes responsible for the deethylation of phenacetin has now been shown to exhibit genetic polymorphism, such that 'poor metabolizers', who have a deficiency or an abnormality in the enzyme, are unable to metabolize phenacetin as readily as the majority of people. Phenacetin can also undergo deacetylation to form p-phenetidine, a reaction catalysed by carboxylesterase. Some recent experimental work suggests that N-hydroxylation of aromatic amino compounds is a key reaction in the formation of metabolites which are responsible for much of their toxicity. It was therefore deemed of interest to examine the metabolic profile of phenacetin in the two different groups in an attempt to correlate one particular pathway with the incidence of nephritis.

Ever since phenacetin has been withdrawn from drugstores in many countries, the main focus of interest seems to have moved from phenacetin to acetaminophen. In some countries, however, there is still a large number of phenacetin abusers and many of them are suffering from interstitial nephritis. To investigate the pathogenesis of the nephropathy, in 1968 Prescott and his coworkers studied the metabolism of phenacetin in man with particular reference to effects on the kidney in healthy volunteers. However they could quantify only unchanged phenacetin and acetaminophen by thin layer chromatography. They could not find any significant differences in the metabolism of phenacetin or acetaminophen. Pharmacokinetics of phenacetin in patients with renal disease was also studied by this group and no significant differences were found from healthy volunteers. Our previous report is in part consistent with it.

In the present study, five metabolites as well as unchanged phenacetin were measured by the HPLC method. In our method the practical limit of sensitivity was 20–50 ng/ml and urinary excretion of the metabolites, including cysteine and mercapturic acid.
acid conjugate were determined. In plasma these two compounds were not detectable, thus the plasma concentration of these substances must be very low.

The values of pharmacokinetic parameters obtained in this study are very similar to those obtained in other studies,\(^\text{14}\) for example the elimination half-life of phenacetin reported shows a variation of between 45 and 90 min. The absorption of phenacetin is highly dependent on formation factors such as particle size,\(^\text{15}\) and in this study as the same brand of phenacetin was used the absorption rate constant was very similar in two groups (0.36±0.09 in group A, 0.34±0.06 in group B, mean±S.D.). The results suggest that the causes of interstitial nephritis in susceptible phenacetin abusers are not associated with enhanced elimination of either phenacetin itself or the metabolites measured in this study. Fig. 2, 3 and 4 show the profiles of these metabolites which are fundamentally similar. Although the size of this study was quite small and it is not therefore possible to conclude definitively that phenacetin is related to the nephritis or not, nevertheless there were no differ-
ences in the pharmacokinetics and metabolic profiles obtained. Therefore changes in the pharmacokinetics and metabolites so far measured are unlikely to be the cause of the interstitial nephritis. However, other minor metabolites such as p-phenetidine might be involved in the pathogenesis of the interstitial nephritis. Particularly in individuals who have a reduced ability to deethylate phenacetin through a genetic deficiency in one, or more, isozymes of cytochrome P-450.

References