Isolation of Perisosaxal Glucuronides and Determination of Enantiomeric Ratios of d- and l-Perisosaxal Metabolites excreted in Rabbit Urine

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Perisosaxal glucuronides were isolated from rabbit urine after repeated subcutaneous administration of dl-perisosaxal citrate. Optical rotatory analysis of free bases obtained from the glucuronides indicated that about twice as much d-perisosaxal glucuronide as l-isomer was formed.

Stereoselective metabolism was observed in rabbit urine after d- or l-perisosaxal administration. d-Perisosaxal glucuronide was excreted predominantly at doses of 1.8 and 9.0 mg/kg. The excretions of d- and l-isomers of p-hydroxyperisosaxal glucuronides were similar at the dose of 9.0 mg/kg, but a larger value was obtained for the l-isomer at the dose of 1.8 mg/kg.

Keywords—3-(1-hydroxy-2-piperidinoethyl)-5-phenylisoxazole; perisosaxal; analgesics; rabbit urine; glucuronide; phenolic metabolites; stereoselective metabolism

Perisosaxal [3-(1-hydroxy-2-piperidinoethyl)-5-phenylisoxazole] possesses analgesic, anti-inflammatory and antitussive activities.1) Previously, phenolic metabolites of perisosaxal were detected in rat urine2) and perisosaxal glucuronide was obtained as one of the major metabolites in man and rabbit.3)

In this study, the isolation of perisosaxal glucuronide and the determination of stereoselective glucuronidation and hydroxylation of d- and l-perisosaxal in rabbit were carried out.

Experimental

Materials—dl-Perisosaxal citrate, o-hydroxyperisosaxal, m-hydroxyperisosaxal and p-hydroxyperisosaxal were synthesized by the same methods as described in the previous paper.3) d-Perisosaxal citrate and l-perisosaxal citrate6) were supplied by Dr. Adachi. Although these perisosaxals were administered as citrate, all doses of the drugs are expressed in terms of the free base.

Animals and Dosing—Commercially obtained male albino rabbits weighing 3.0–4.5 kg were used. For the isolation of perisosaxal glucuronide, 22.2 mg/kg of dl-perisosaxal citrate dissolved in 0.5% NaCl was administered subcutaneously to three rabbits at the dose of 66.5 mg/kg once a day for 6 d. The urine was collected 24 h after each dosing. In order to examine the stereoselectivity of formation of perisosaxal glucuronides and hydroxylated metabolites excreted in the urine, d- or l-perisosaxal citrate was administered intravenously at doses of 1.8 and 9.0 mg/kg. The urine was collected until 4 h after dosing via a catheter inserted into the blader.

Isolation of Perisosaxal Glucuronides and the Free Base—The isolation of perisosaxal glucuronides was performed as shown in Fig. 1. The pooled urine was filtered and loaded onto an Amberlite XAD-2 resin (Rohm and Haas) column (5 cm × 45 cm) previously washed with 10 l of water, then eluted with 2.5 l of methanol. The methanolic eluate from the 7th to 12th fractions (200 ml/fraction) was evaporated to dryness at 40°C under reduced pressure. The residue was dissolved in 50 ml of water, and the solution was diluted with 120 ml of methanol, then applied to 22 preparative thin layer chromatography (TLC) plates (20 cm × 100 cm, 0.75 mm thickness, Silica gel F254, Merck) and developed with chloroform–methanol–water–acetic acid (13: 10: 3: 1). The silica gel powder around Rf 0.45 (perisosaxal glucuronides) was scraped off the plates and extracted with methanol. The methanolic extract was chromatographed with methanol on an Amberlite IRC-50 resin (Rohm and Haas) column (5 cm × 40 cm). Contaminating free base was absorbed by the resins.8) The effluent was evaporated to dryness at 40°C under reduced pressure. The residue was crystallized from small amounts of methanol to give perisosaxal glucuronides as a white powder. Anal. Caled for C_{76}H_{166}N_{12}O_{6}: C, 58.52; H, 6.29; N, 6.24. Found: C, 58.54; H, 6.33; N, 6.13.
filtered urine
  \[\text{Amberlite XAD-2}\]
methanol eluate
  preparative TLC\textsuperscript{a)}
  \[
  \begin{array}{l}
  \text{Silica gel GF}_{254} \\
  \text{CHCl}_3-\text{methanol-H}_2\text{O-AcOH} \quad (13: 10: 3: 1)
  \end{array}
  \]
methanol extract
  \[\text{Amberlite IRC-50}\textsuperscript{b)}\]
methanol eluate
  residue
  crystallize from methanol
  \[
  \begin{array}{l}
  \text{crystals}\textsuperscript{c)} \\
  (\text{fraction I}) \quad \text{mother liquor} \quad \text{evaporate} \quad \text{residue}\textsuperscript{d)} \\
  (\text{fraction II}) \quad \text{hydrolyze with} \\
  \beta\text{-glucuronidase,} \quad \text{extract with} \\
  \text{cyclohexane} \\
  \text{residue}\textsuperscript{c)} \\
  (\text{fraction III}) \quad \text{hydrolyze with} \\
  \beta\text{-glucuronidase,} \quad \text{extract with} \\
  \text{cyclohexane} \\
  \text{residue}\textsuperscript{c)} \\
  (\text{fraction IV})
  \end{array}
  \]

Fig. 1. Isolation of Isomeric Perisoxal Glucuronides from Rabbit Urine after Subcutaneous Administration of dl-Perisoxal Citrate

Urine samples from three rabbits were collected (54 h samples) after each dosing. The dose was 66.5 mg/kg once a day for 6 d.

\textit{a) A mixture of d- and l-perisoxal glucuronides was separated from other metabolites and urine components.}
\textit{b) Contaminating compounds were removed from the glucuronides.}
\textit{c) A mixture of d-perisoxal glucuronide and l-perisoxal glucuronide.}
\textit{d) A mixture of d-perisoxal and l-perisoxal.}

\textbf{Results and Discussion}

\textbf{Isolation of Perisoxal Glucuronides and Determination of Enantiomeric Ratio after Subcutaneous Administration of dl-Perisoxal Citrate to Rabbits}

Excretion of perisoxal glucuronide in rabbits was greater than that of rats, e.g., about 10\% of the dose.\textsuperscript{3)} Therefore perisoxal glucuronides were isolated from rabbit urine after repeated subcutaneous administration of dl-perisoxal citrate.

The glucuronides isolated and purified as described above resisted HCl hydrolysis, but were almost completely hydrolyzed with \(\beta\)-glucuronidase to give a single spot of perisoxal on TLC. Stereoselective hydrolysis of d- and l-oxazepam glucuronides by \(\beta\)-glucuronidase preparation has been reported,\textsuperscript{6)} but in our experiment fraction I, which contained mostly d-perisoxal...
TABLE I. Enantiomeric Composition of Perisoxal Glucuronide and Perisoxal in Rabbit Urine after Subcutaneous Administration of dl-Perisoxal Citrate

<table>
<thead>
<tr>
<th>Compound</th>
<th>([\chi]_D^0) (CHCl_3) or amount</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>dl-Perisoxal</td>
<td>+40.2°</td>
<td></td>
</tr>
<tr>
<td>l-Perisoxal</td>
<td>-40.1°</td>
<td></td>
</tr>
<tr>
<td>Fraction III</td>
<td>+36.8°</td>
<td>d-Perisoxal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95.8%</td>
</tr>
<tr>
<td>Fraction IV</td>
<td>+4.3°</td>
<td>l-Perisoxal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.2%</td>
</tr>
<tr>
<td>Fraction I</td>
<td>196 mg</td>
<td>d-Perisoxal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>glucuronide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>188 mg</td>
</tr>
<tr>
<td>Fraction II</td>
<td>387 mg</td>
<td>l-Perisoxal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>glucuronide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 mg</td>
</tr>
</tbody>
</table>

Dosing conditions were as described in Fig. 1.

Glucuronide and some l-perisoxal glucuronide, and fraction II, which contained about equal amounts of these glucuronides, were hydrolyzed to similar extents of 86 and 88%, respectively. Thus, no enantiomeric difference was apparent in the hydrolysis.

The optical rotations of fraction III (free perisoxals from fraction I) and fraction IV (free perisoxals from fraction II) were measured, then the amount of each enantiomeric compound was calculated (Table I). The amounts of d- and l-perisoxal glucuronides were 188 and 8 mg in fraction I and 214 and 173 mg in fraction II, respectively. Therefore, the total ratio of d- to l-perisoxal glucuronide was d-(188 mg + 214 mg): l-(8 mg + 173 mg) = 2.2:1. Thus, stereoselective glucuronidation of perisoxal in rabbits was confirmed.

**Urinary Excretion of Metabolites after Intravenous Administration of d- or l-Perisoxal Citrate to Rabbits**

For further investigation of the stereoselective metabolism of perisoxal in rabbits, perisoxal glucuronides and hydroxylated metabolites following intravenous administration of d- or l-perisoxal citrate were determined. As shown in Table II, there was some variation in the excretion of unchanged perisoxal, ranging from 1.7% to 7.97%, for doses of 1.8 and 9.0 mg/kg. The excretions of glucuronide of d-perisoxal were larger than those of glucuronide of the l-isomer, clearly demonstrating an enantiomeric difference in the glucuronidation of the parent drugs.

Phenolic metabolites were excreted as glucuronides and not as free bases. The position of conjugation of phenolic metabolites with glucuronic acid was considered to be the phenolic

**TABLE II. Urinary Excretions of Perisoxal and Its Phenolic Metabolites in Rabbits after Intravenous Administration of d- or l-Perisoxal Citrate**

<table>
<thead>
<tr>
<th>Drug and dose</th>
<th>Perisoxal (%)</th>
<th>Perisoxal glucuronide (%)</th>
<th>Hydroxyperisoxal glucuronide (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d- 9.0 mg/kg</td>
<td>6.38</td>
<td>16.14</td>
<td>0.13</td>
</tr>
<tr>
<td>l- 9.0 mg/kg</td>
<td>7.13</td>
<td>20.17</td>
<td>0.09</td>
</tr>
<tr>
<td>d- 1.8 mg/kg</td>
<td>7.97</td>
<td>9.01</td>
<td>0.13</td>
</tr>
<tr>
<td>l- 1.8 mg/kg</td>
<td>3.85</td>
<td>4.15</td>
<td>0.07</td>
</tr>
<tr>
<td>d- 1.8 mg/kg</td>
<td>7.91</td>
<td>18.34</td>
<td>n.d.</td>
</tr>
<tr>
<td>l- 1.8 mg/kg</td>
<td>1.70</td>
<td>20.23</td>
<td>n.d.</td>
</tr>
<tr>
<td>d- 1.8 mg/kg</td>
<td>5.52</td>
<td>5.39</td>
<td>n.d.</td>
</tr>
<tr>
<td>l- 1.8 mg/kg</td>
<td>4.16</td>
<td>5.23</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

The urine was collected for 4 h after dosing via a catheter inserted into the bladder.

a) Free base equivalent. n.d.: not detected.
OH, since they were easily hydrolyzed with HCl, liberating almost the same amounts of free base as with β-glucuronidase, while the glucuronide of the intact drug, in which the molecular site of conjugation seemed to be the alcoholic OH, was difficult to hydrolyze with HCl as described above. In the glucuronides of hydroxyperisoxals, the excretion of β-hydroxyperisoxal glucuronide was largest, followed by that of m-hydroxyperisoxal. Very little o-hydroxyperisoxal glucuronide was excreted. The excretion of β-hydroxyperisoxal glucuronide of the L-isomer was larger than that of the d-isomer at the dose of 1.8 mg/kg, but almost the same values were obtained at the dose of 9.0 mg/kg. Excretions of m-hydroxyperisoxal glucuronide of the d-isomer were larger than those of the L-isomer, but these differences may not be significant, since the values were small (about 1—3% of the dose).

In this experiment, the number of rabbits used was only two in any dosage regimen; nevertheless, the percentages of excreted metabolites in the urines of the two rabbits were in good accord. Therefore it is considered that the glucuronidation of d- and L-perisoxal was stereoselective at both 1.8 and 9.0 mg/kg doses, but the β-hydroxylation, followed by glucuronidation, was apparently stereoselective only at the 1.8 mg/kg dose. Therefore, the stereoselectivity of the β-hydroxylation of d- and L-perisoxal in rabbits may be dose-dependent. To confirm this, an experiment with a smaller dose than 1.8 mg/kg is desirable, but could not be performed here because of the inadequate sensitivity of the analytical method used by us.

References and Notes


