Pharmacokinetics and Biotransformation of Beraprost Sodium V: Plasma Level Profile of Beraprost Sodium in Dog

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Summary

The plasma levels of beraprost sodium and its major metabolites were determined in dogs after oral administration of 3H-labeled beraprost sodium.

The major metabolites in the dog plasma were found to be 13, 14-dihydro-15-oxo-beraprost, 2,3-dinor-beraprost and 13, 14-dihydro-2,3-dinor-15-oxo-beraprost as determined by co-chromatography with synthetic reference standards on high-performance liquid chromatography. The plasma concentration of unchanged beraprost sodium increased rapidly, reaching a maximum concentration 20 to 25min (mean value) in males and 10 to 24min (mean value) in females after dosing of 0.008, 0.04 and 0.2mg/kg, and then declined thereafter with a biphasic pattern. The metabolites appeared in the plasma rapidly after dosing and the major metabolite was 13, 14-dihydro-15-oxo-beraprost in both males and females.
Introduction

Beraprost sodium (TRK-100: sodium (±)-(1$^R*$, 2$^R*$, 3a$^S*$, 8b$^S*$)-2, 3, 3a, 8b-tetrahydro-2-hydroxy-1-[(E)-3$^S*$-3-hydroxy-4-methyl-1-octen-6-ynyl]-1H-cyclopenta[b]-benzofuran-5-butylate) is a new and orally active prostacyclin derivative having a potent inhibitory effect on platelet aggregation with a vasodilating activity\(^1,2\).

In the preceding studies\(^3\)\(^\rightarrow\)\(^6\), we have revealed that there is a sex difference in pharmacokinetics and metabolism of beraprost sodium in rats. Therefore, in order to further clarify the feature of beraprost sodium, we examined plasma levels of beraprost sodium and its major metabolites in both male and female dogs after oral administration of $^3$H-lebeled beraprost sodium.

Materials and Methods

1. Chemicals

Beraprost sodium (BPS) was synthesized

\begin{align*}
\text{COOH} & \quad \text{COOH} & \quad \text{COOH} \\
\text{OH} & \quad \text{OH} & \quad \text{OH} \\
\text{CH}_3 & \quad \text{CH}_3 & \quad \text{CH}_3 \\
\text{CH}_3 & \quad \text{CH}_3 & \quad \text{H}
\end{align*}

in the Basic Research Laboratories of Toray. Tritium-labeled beraprost sodium ($^3$H-beraprost sodium) was synthesized and supplied by the New England Nuclear (USA) as described in the previous paper\(^3\). The chemical structure of beraprost sodium and the labeling position are presented in Fig. 1. The following reference standards (Fig. 2) were synthesized in the Basic Research Laboratories of Toray: 2,3-dinor-beraprost (D1/D2), 13,14-dihydro-2,3-dinor-15-oxoberaprost (P2), 13,14-dihydro-15-oxoberaprost (P3), 15-epi-2,3-dinor-bera-prost (C

Fig. 1 Chemical structure of beraprost sodium and labeling position

Fig. 2 Chemical structures of synthetic reference standards
3, $\alpha$-3, 4-didehydro-beraprost(E2), APS-308. APS-308 labeled with $^3$H at C-15 position ($^3$H-APS-308) was synthesized in the Basic Research Laboratories of Toray. The specific radioactivities of $^3$H-beraprost sodium and $^3$H-APS-308 were 46.8mCi/mg and 0.199mCi/mg, respectively. The radiochemical purity was more than 98% by thin-layer chromatography (TLC) for $^3$H-beraprost sodium and by high-performance liquid chromatography (HPLC) for $^3$H-APS-308. All other reagents and organic solvents were of analytical grade.

2. Animals and dosing

Male and female beagle dogs weighing 8 to 11kg were used. The dogs were housed in an animal room maintaining a temperature at 23-27°C and a relative humidity of 45-65% with 12hr-light cycle. They were fed with a solid food (CD-1, Clea Japan) twice a day (120g each) and tap water was given ad libitum. The dogs were fasted for 18hrs before and 10hrs after dosing.

$^3$H-Beraprost sodium was mixed with unlabeled beraprost sodium, if necessary, and dissolved in distilled water. Dose levels were set at 0.008, 0.04 and 0.2mg/kg and the concentration of the dosing solutions were approximately 0.016mg/0.75μCi/ml, 0.08mg/3.74μCi/ml and 0.40mg/3.74μCi/ml. The drug was administered as a gelatine capsule containing a dosing solution.

3. Collection of plasma samples

Beagle dogs (four males or four females/group) received an oral dose of $^3$H-beraprost sodium and blood samples (1-5 ml/each) were collected from a cephalic vein at 5, 10, 30, 45min, 1, 2, 6, 10 (0.04 and 0.2mg/kg only) and 24hrs after dosing. The blood samples were subsequently centrifuged (3,000rpm, 10min) at 10°C and the resulting plasma samples were used for the analysis of metabolites.

4. Analysis of metabolites in plasma

The metabolites were determined by co-chromatography with the synthetic reference standards on HPLC in the same way as described in the previous paper. To plasma samples were added $^3$H-APS-308 as an internal standard and the synthetic reference standards as carriers. The resulting mixture was acidified with 1N hydrochloric acid and then treated with SEP-PAK C18 cartridge (Waters Associates). After washing with water, radioactivity in the cartridge was eluted with methanol and the methanol eluates were subjected to HPLC analysis.

5. Measurement of radioactivity

Radioactivity in the plasma and eluates from a HPLC column was determined using liquid scintillation counters (Packard Tri-Carb 1500 or LKB 1218) as described in the previous report.

6. Pharmacokinetic analysis

Values of Cmax and Tmax were directly read from concentration data. Values of area under the concentration-time curve (AUC) were calculated by trapezoidal method. Half-lives were determined by a nonlinear regression analysis. The concentrations were best fitted by 2-compartment model.

Results

1. Metabolite compositions in plasma

Fig. 3 represents typical patterns of the plasma metabolites after oral administration of $^3$H-beraprost sodium to dogs. Beraprost sodium is a mixture of two isomers which can be separated by HPLC as shown in the chromatogram. The unchanged drug as well as three major metabolites, P2, P3 and D1/D2 were observed in the plasma, together with a number of minor component of metabolites. Thirty
min after oral dosing of 0.04 mg/kg, for example, beraprost sodium and these major metabolites accounted for 71% of the total plasma radioactivity in males and 60% in females. P3, P2 and D1/D2 were predominant even 6 hrs after dosing, accounting for 48% and 36% of the total plasma radioactivity in males and females, respectively, whereas the unchanged beraprost sodium decreased to the level of approximately 1% of the total radioactivity.

2. Plasma concentration of unchanged drug

Fig. 4 and Table I show the plasma level profiles of the unchanged beraprost sodium and pharmacokinetic parameters after oral administration of $^3$H-beraprost sodium to dogs at doses of 0.008, 0.04 and 0.2 mg/kg.

In males, plasma concentration of the

Fig. 3 Typical radiochromatograms of plasma samples after oral administration of $^3$H-beraprost sodium to dogs
unchanged beraprost sodium increased rapidly, reaching a maximum concentration (Cmax) 20±10, 20±12 and 20±12 min after dosing of 0.008, 0.04 and 0.2 mg/kg, respectively. The maximum concentrations of 0.875, 3.30 and 10.45 ng/ml were achieved at three doses, respectively. The plasma concentrations declined thereafter, showing a biphasic pattern with half-lives of 0.20 to 0.74 hrs (α-phase) and 3.2 to 4.7 hrs (β-phase). Twenty-four hrs after dosing, the plasma concentrations decreased to the levels of approximately 1% of the Cmax. The area under plasma level-time curves (AUC) were 0.57 (0-6 hr), 3.0 (0-24 hr) and 13.2 ng•hr/ml (0-24 hr), respectively.

In females, plasma levels of the unchanged beraprost sodium after oral dosing

![Fig. 4 Concentration of beraprost sodium in plasma after oral administration of ³H-beraprost sodium to dogs (mean ± s.d., n=4)](image)

*) The data derived from pooled plasma samples of four dogs were used as the mean values,
Table I Pharmacokinetic parameters of beraprost sodium after oral administration of 3H-beraprost sodium to dogs

<table>
<thead>
<tr>
<th>Sex</th>
<th>Dose (mg/kg)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (min)</th>
<th>AUC&lt;sub&gt;C&lt;sub&gt;0&lt;/sub&gt;→24hr&lt;/sub&gt; (ng·hr/ml)</th>
<th>T&lt;sub&gt;1/2α&lt;/sub&gt; (hr)</th>
<th>T&lt;sub&gt;1/2β&lt;/sub&gt; (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0.008</td>
<td>0.875±0.224&lt;sup&gt;1)&lt;/sup&gt;</td>
<td>25±10</td>
<td>0.572&lt;sup&gt;2)&lt;/sup&gt;</td>
<td>0.20</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>3.30±0.52</td>
<td>20±12</td>
<td>3.0</td>
<td>0.22</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>10.45±4.19</td>
<td>20±12</td>
<td>13.2</td>
<td>0.74</td>
<td>4.7</td>
</tr>
<tr>
<td>Female</td>
<td>0.008</td>
<td>1.40±0.59</td>
<td>24±17</td>
<td>0.88</td>
<td>0.25</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>6.20±4.43</td>
<td>14±11</td>
<td>4.4</td>
<td>0.24</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>14.1±4.20</td>
<td>10±0</td>
<td>16.6</td>
<td>0.68</td>
<td>3.9</td>
</tr>
</tbody>
</table>

<sup>1)</sup> mean ± s.d.
<sup>2)</sup> AUC<sub>C<sub>0</sub>→24hr</sub>

Table II Pharmacokinetic parameters of metabolites after oral administration of 3H-beraprost sodium to dogs

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Dose (mg/kg)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (min)</th>
<th>AUC&lt;sub&gt;C&lt;sub&gt;0&lt;/sub&gt;→24hr&lt;/sub&gt; (ng·hr/ml)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (min)</th>
<th>AUC&lt;sub&gt;C&lt;sub&gt;0&lt;/sub&gt;→24hr&lt;/sub&gt; (ng·hr/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P3</td>
<td>0.008</td>
<td>0.947±0.147&lt;sup&gt;1)&lt;/sup&gt;</td>
<td>34±8</td>
<td>1.63</td>
<td>0.911±0.211</td>
<td>38±15</td>
<td>1.50</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>3.78±0.78</td>
<td>34±8</td>
<td>6.0</td>
<td>3.76±1.01</td>
<td>30±0</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>10.5±1.0</td>
<td>45±0</td>
<td>23.2</td>
<td>13.5±5.4</td>
<td>38±9</td>
<td>24.2</td>
</tr>
<tr>
<td>D1/D2</td>
<td>0.008</td>
<td>0.592±0.214</td>
<td>34±8</td>
<td>1.44</td>
<td>0.710±0.215</td>
<td>38±15</td>
<td>1.95</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>2.14±0.35</td>
<td>34±8</td>
<td>6.5</td>
<td>2.44±0.46</td>
<td>38±15</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>6.20±1.23</td>
<td>34±8</td>
<td>28.6</td>
<td>8.56±1.16</td>
<td>30±0</td>
<td>43.5</td>
</tr>
<tr>
<td>P2</td>
<td>0.008</td>
<td>0.817±0.573</td>
<td>41±8</td>
<td>2.87</td>
<td>0.651±0.420</td>
<td>41±14</td>
<td>3.06</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>2.96±1.72</td>
<td>41±8</td>
<td>13.6</td>
<td>2.20±1.43</td>
<td>45±12</td>
<td>12.1</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>8.45±5.11</td>
<td>38±9</td>
<td>54.0</td>
<td>8.19±5.10</td>
<td>45±0</td>
<td>57.0</td>
</tr>
</tbody>
</table>

<sup>1)</sup> mean ± s.d.

were nearly identical with those in males, although C<sub>max</sub> and AUC were slightly higher in females than in males.

3. Plasma concentration of metabolites

Fig. 5 and Table II show the plasma levels and pharmacokinetic parameters of the major metabolites after oral administration of 3H-beraprost sodium to dogs at doses of 0.008, 0.04 and 0.2mg/kg.

In males, plasma concentration of metabolites P3, P2 and D1/D2 increased in parallel with a rise in the concentration of beraprost sodium. The major metabolites were found to be P3, P2 and D1/D2 at every doses examined. The plasma concentrations of these metabolites declined thereafter in a manner similar to that of beraprost sodium.

In females, plasma levels of P3, P2 and D1/D2 after oral administration of 3H-beraprost sodium were similar to those in males.

Discussion

In male and female dogs, plasma levels of the unchanged drug increased rapidly after oral administration of 3H-beraprost sodium and the C<sub>max</sub> was observed at 20 to 25min (mean value) in males and 10 to 24min(mean value) in females after dosing
of 0.008, 0.04 and 0.2 mg/kg, indicating rapid absorption of the drug from a gastrointestinal tract. An increase in Cmax and AUC was almost dose-related at doses of 0.008 and 0.04 mg/kg. However, an increase of AUC was not proportional to dose level and prolongation of half-lives of α-phase was observed in both male and female dogs at a dose of 0.2 mg/kg. Although there is no direct evidence, this may be due in part to an inhibitory effect of beraprost sodium on gastric emptying which is observed at higher dose than 0.2 mg/kg\(^4\). The main metabolites in the dog plasma were found to be 13, 14-dihydro-15-oxo-beraprost (P3), 2,3-dinor-beraprost (D1/D2) and 13, 14-dihydro-2,3-dinor-15-oxo-beraprost (P2), all of which have been observed in the rat plasma\(^3\). The major metabolite in the plasma was P3 in both
males and females. There is no significant difference between males and females in the Cmax of unchanged beraprost sodium, although plasma levels of beraprost sodium were slightly higher in females than in males.

Comparing the metabolite composition in plasma between dogs and rats, the most remarkable difference is seen in the amount of 13,14-dihydro-15-oxo compounds, namely P2 and P3, which are minor metabolites in rat plasma. It has been well established that except for Prostaglandin I2 (PGI2), natural prostaglandins are metabolized by the oxidation of C-15 hydroxy group with 15-hydroxyprostaglandin (15-OH-PG) dehydrogenase in lungs followed by the reduction of the 13,14-double bond with A13-reductase in liver and kidneys. PGI2, however, is known to be resistant to this deactivation by 15-OH-PG dehydrogenase because of its low affinity to the pulmonary prostaglandin transport system of rats. Furthermore, it has been reported that an introduction of alkyl group(s) at C-16 position protects the C-15 hydroxy group against the oxidation by 15-OH-PG dehydrogenase of rats. Therefore, beraprost sodium, a PGI2 analogue with a monomethyl group at C-16 position, is thought to be less efficient substrate for 15-OH-PG dehydrogenase. In fact, only small amounts of 13,14-dihydro-15-oxo compounds, P2 and P3, are present in the rat plasma, in which β-oxidation product, D1/D2 is the major metabolite. This study showed that 13,14-dihydro-15-oxo compounds were present in the dog plasma to a large extent. This result suggests that the 15-OH-PG dehydrogenase activity is higher in dogs than in rats, although there are scanty reports on the metabolism of natural PGI2 in dogs.

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References
