Studies on the Metabolic Fate of Pramipexole (SND 919 CL₂Y) (II):
Absorption and Distribution after Repeated Oral Administration to Rats

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Summary: The absorption and distribution of radioactivity were investigated following a 14-day period of
daily oral administration of ¹⁴C-pramipexole (0.5 mg/kg/day) to male rats.

1. When measured 24 hr after each of 14 repeated daily administrations of ¹⁴C-pramipexole to male
rats, the level of radioactivity in plasma rose as the number of doses increased, and reached a steady state af
ter 12 or 13 doses. The elimination of radioactivity from the plasma after the last dose was similar to that af
ter a single administration.

2. At 1 hr after the 14th administration, the level of radioactivity reached the maximum in most tissues.
High levels were observed in the liver and kidney, being about 22 and 14 times higher than that in the plas-
ma, respectively. At 168 hr after the last dose, the levels of radioactivity in the liver and kidney were very
much higher than that in plasma. The elimination of radioactivity from most tissues after the last dose was
parallel to that from plasma, except the spleen and kidney, where an accumulation was observed.

3. After the last administration, the ratio of the concentration in blood cells to that in plasma gradually
increased with the lapse of time. The elimination of radioactivity from blood cells was very much slower
than that from plasma. The relative increase of radioactivity in blood cells at the late stage after drug ad-
ministration may be attributed to metabolites of pramipexole.

Key words: Pramipexole, Absorption, Distribution, Excretion, Rats, Repeated administration

Introduction

Pramipexole dihydrochloride (abbreviated as pramipexole) is a synthetic benzothiazole derivative. It
binds to pre- and postsynaptic dopamine D₂ and D₃ recep-
tors but has the highest affinity for dopamine D₃ recep-
tor subtype.¹⁻⁴) It is currently being developed for the
administration of the signs and symptoms of idiopathic Par-
kinson’s disease.

We have previously reported the absorption, distribu-
tion, and excretion of radioactivity after a single oral ad-
mistration of ¹⁴C-pramipexole to rats.⁵) In the present
study, the absorption and distribution of radioactivity
were investigated after repeated oral administration of
¹⁴C–labeled pramipexole to rats at a dose of 0.5 mg/kg
once a day for 14 days.

Material and Methods

1. Labeled Compound

¹⁴C–Pramipexole (Batch No. 16 and 17) and unla-
beled pramipexole (Batch No. II) were synthesized at
Boehringer Ingelheim KG (Germany). The specific ac-
tivity was 6700 kBq/mg and the radiochemical purity
was more than 96% as determined by thin-layer chro-
matography (TLC). The structure and labeled position
were shown in the preceding paper.⁵)

2. Animals

Sprague-Dawley strain SPF male rats (obtained from
Japan S.L.C.) were used at 8 weeks of age. The rats
were acclimatized before use as described previously.⁵)

The rats were allowed free access to food and water
throughout the study.

3. Administration of the Drug

¹⁴C–Pramipexole mixed with unlabeled pramipexole
was dissolved in distilled water to give a final concen-
tration of 0.05 mg/ml. Each rat received the drug solution
as an oral dose equivalent to 0.5 mg/kg once a day for 14
days via a stomach tube.

4. Plasma Concentration of Radioactivity

After oral administration of ¹⁴C-pramipexole to non-
fasted male rats, the rats were anesthetized with ethyl
ether, and blood samples were taken from the retro-or-
bital venous plexus into heparinized tubes and cen-
trifuged immediately to obtain the plasma.

Sampling time points were as follows:
Day 1: 0.5, 2, 6, and 8 hr after administration
Days 2, 4, 7, 9, 12, 13, and 14: immediately before ad-
ministration

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309
Day 14: 0.25, 0.5, 1, 2, 4, 6, 8, 24, 48, 72, 96, and 168 hr after administration.

The concentration of radioactivity in plasma was determined by use of a liquid scintillator (Hionic Fluor; Packard, USA) as described previously.5)

5. Tissue Concentration of Radioactivity
At 1, 6, 24, 96, 168, 240, and 336 hr after the completion of the 14-day repeated oral administration of 14C-pramipexole to four rats for each time point, the tissues and organs were removed and weighed. The radioactivity in the plasma, blood, tissues and organs was measured as described previously.5)

The hematocrit value was measured and the concentration of radioactivity in the blood cells was calculated as described previously.5)

6. Calculation of Pharmacokinetic Parameters
The concentrations of total radioactivity in plasma were expressed as ng equivalent of pramipexole per ml. The elimination half-life (t1/2) after the completion of the 14-day repeated oral administration was calculated from the log-linear part of the plasma concentration vs. time curve. The area under the plasma concentration-time curve up to the final measurement time (AUC0-t) after completion of the 14-day repeated oral administration was calculated by the trapezoidal method. The mean residence time (MRT) was calculated by the moment method.

7. Measurement of Radioactivity
Radioactivity in each test sample was measured in a liquid scintillation spectrometer (Tri-Carb 2500 TR or 2700 TR; Packard, USA). Quenching was corrected by means of the external standard method. Concentrations of radioactivity were expressed as equivalents of pramipexole.

Results
1. Plasma Concentration of Radioactivity
Mean concentrations of total radioactivity in plasma during and at every 24 hr after oral administration of 14C-pramipexole to rats at a dose of 0.5 mg/kg once a day for 14 days are shown in Fig. 1. The concentrations of radioactivity in plasma after a single administration5) and the 14th repeated administration are shown in Fig. 2, and the calculated pharmacokinetic parameters after the last (14th) administration are summarized in Table I. The calculated pharmacokinetic parameters after single administration5) are also given in Table I, for comparison.

The plasma concentration of total radioactivity measured at 24 hr after daily oral dosing increased with increasing number of doses and tended to reach a steady state by day 12 or 13.

After the last administration, the concentration reached a maximum (Cmax) of 77.0 ng eq./ml at 1.2 hr and showed a half-life of about 51 hr between 24 and 72 hr. The half-life calculated from the same time points between 24 and 72 hr was the same that as after single administration to non-fasted rats (47.1 hr).5)

The accumulation factor calculated from mean Cmin(12th dose) or Cmin(13th dose)/mean Cmin(1st dose) was 3.79 or 3.89, respectively. The accumulation factor calculated from the equation 1/(1-e^(-λt)), where λ is the elimination rate constant after single administration in non-fasted rats (t1/2 is 47.1 hr5) and r is the dosing interval (24 hr), was 3.36.

![Fig. 1 Plasma concentration of total radioactivity at 24 hr after daily oral administration of 14C-pramipexole to male rats at a dose of 0.5 mg/kg once a day for 14 days](image-url)

Data are expressed as the mean±S.D. of five animals.
Fig. 2 Plasma concentration of total radioactivity after single administration and after the completion of 14-day repeated oral administration of $^{14}$C-pramipexole to male rats at a dose of 0.5 mg/kg. Data are expressed as the mean ± S.D. of five animals.

Table I Pharmacokinetic parameters of total radioactivity in plasma after single administration and after the completion of 14-day repeated oral administration of $^{14}$C-pramipexole to male rats at a dose of 0.5 mg/kg

<table>
<thead>
<tr>
<th>Parameter</th>
<th>single administration</th>
<th>after 14-day administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$ (ng eq./ml)</td>
<td>28.8±4.6</td>
<td>77.0±9.2</td>
</tr>
<tr>
<td>$t_{max}$ (hr)</td>
<td>1.1±0.5</td>
<td>1.2±0.4</td>
</tr>
<tr>
<td>$t_{1/2}(24-72)$ (hr)</td>
<td>47.1±5.4</td>
<td>51.2±5.7</td>
</tr>
<tr>
<td>AUC$_{(0-72)}$ (ng eq.·hr/ml)</td>
<td>832.2±30.9</td>
<td>2696.0±248.2</td>
</tr>
<tr>
<td>MRT$_{(0-72)}$ (hr)</td>
<td>27.2±1.8</td>
<td>28.7±0.7</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± S.D. of five animals.

2. Tissue Concentrations of Radioactivity

Mean concentrations of total radioactivity in the organs and tissues up to 168 hr after repeated oral administration of $^{14}$C-pramipexole at 0.5 mg/kg once a day for 14 days to male rats are shown in Table II. The concentrations of radioactivity in the tissues showed maxima at 1 hr, except for the blood, prostate, testis and large intestine with contents, which showed maxima at 6 hr. At this time, high concentrations were observed in the liver and kidney, which contained about 22 and 14 times higher radioactivity than the plasma, respectively, followed by the lung, spleen and salivary gland, which contained 4.5 to 5.7 times higher radioactivity than the plasma. The concentrations in the tissues except the adipose tissue, testis and eyeballs were higher than that in the plasma.

High concentrations were observed in the liver, kidney and lung, which contained about 27, 13, and 4.5, and 33, 30, and 6.4 times higher level of radioactivity than the plasma at 24 and 168 hr after administration, respectively.

The half-life of radioactivity in the plasma, calculated from the data obtained from 24 to 168 hr, was about 82 hr. Half-lives of radioactivity in the skeletal muscle (about 181 hr), trachea (about 186 hr), eyeballs (about 194 hr), spleen (about 256 hr), blood (about 273 hr) and kidney (about 278 hr) were 2 to 3 times longer than that of plasma. However, the other tissues showed half-lives of less than twice that in plasma.

The level of total radioactivity in the spleen, kidney and blood were very much higher than that in plasma at 168 hr after administration, and moreover the half-lives of radioactivity in them were longer than that in plasma. To investigate the elimination of radioactivity from them after 168 hr, we performed an additional tissue distribution study after a 14-day period of daily oral administration.

The results are shown in Table III. The concentrations in the liver, kidney and spleen were about 38, 29 and 6.6 times higher than that in plasma at 168 hr after the last administration, being similar to those described above. Thereafter, the concentrations in the tissues decreased slowly, and were about 42, 40, and 8.5, and 55, 66, and 16 times higher than that in plasma at 240 and 336 hr after the last administration, respectively.

3. Distribution of Radioactivity to Blood Cells

The distribution of radioactivity in the blood cells after repeated oral administration of $^{14}$C-pramipexole at 0.5 mg/kg once a day for 14 days to male rats is shown in Table IV. The ratio of the concentration in blood cells to that in plasma was in the range of 4.99–26.64 at 1–
168 hr. After the last dosing, the ratio gradually increased with the lapse of time. Furthermore, the distribution of radioactivity in the blood cells based on the additional tissue distribution study is shown in Table V. At 168 hr after the last administration, the ratio of the concentration in blood cells to that in plasma was the same as described above. The ratio gradually increased with the lapse of time.

**Discussion**

The concentration of plasma radioactivity in rats was investigated during and after 14 doses of $^{14}$C-
pramipexole administered daily (Fig. 1). A steady state was essentially achieved by the 12th or 13th dosing. The accumulation factor (3.79 or 3.89) calculated from Cn,;, was similar to the theoretical value (3.36) calculated from the half-life after single administration, and the tmax (1.2±0.4 hr) and half-life (51.2±5.7 hr) after the last administration were similar to those after single administration (tmax: 1.1±0.5 hr, t1/2: 47.1±5.4 hr). In general, AUC(0-7) during a dosing interval (r) in the steady state is the same as the AUC(0-7) after a single dose. In the present study, the AUC(0-7) (1055.8±130.0 ng eq. • hr/ml) of the first dose calculated from the half-life after single administration (t1/2: 47.1 hr) was not much different from the AUC (0-24) (1241.1 ± 127.9 ng eq. • hr/ml) of the 14th dose. Therefore, neither induction nor inhibition of enzyme reaction seemed to occur, in terms of the plasma radioactivity concentration profile, and the pharmacokinetics of pramipexole seems not to be affected by repeated administration.

The distribution and elimination of tissue radioactivity was investigated after 14 daily doses of 14C pramipexole. At 168 hr after the last dose, the concentrations of radioactivity in the liver, kidney, blood, lung and spleen were higher than that in plasma. Similar findings were obtained in a study with talipexole, which is used for the treatment of Parkinson’s disease. The elimination of radioactivity from most tissues, except the skeletal muscle, trachea, eyeballs, spleen, blood and kidney, which showed slower elimination, was parallel to that from plasma. Thus, accumulation was observed in the spleen, kidney and blood. Concerning the concentration of radioactivity in the spleen, the mean concentration of radioactivity at 168 hr was higher than that at 96 hr (Table II). This may be due to inter-individual differences. We further investigated the elimination of radioactivity from the spleen, kidney and blood after 168 hr, after a 14 -day period of daily oral administration (Table III). At 168 hr, the tissue/plasma ratios of the liver (reference organ), kidney and spleen were similar to those found in the above-mentioned distribution study. After 168 hr, remarkably slow elimination of radioactivity from spleen (t1/2: about 82 hr, calculated from data for 168 to 336 hr) and kidney (t1/2: about 551 hr) was observed, while the elimination of radioactivity from liver (t1/2: about 172 hr) was almost parallel to that from plasma (t1/2: about 111 hr). The observations in this study indicate the accumulation of radioactivity in the spleen and kidney. Pramipexole was metabolized to several metabolites (data not shown). An N-despropyl metabolite and/or a hydroxy metabolite might be concerned with the accumulation of radioactivity in the spleen and kidney.

In the toxicity studies of pramipexole, however, no histopathological change was seen in the spleen or kidney in a 52-week toxicity study in rats (data not shown). The half-lives of radioactivity were calculated from the blood cell and plasma concentration-time profiles between 24 to 168 hr (Table IV), and values of about 381 and 82 hr were estimated, respectively. The half-life in blood cells was much longer than that in plasma, indicating a tendency to accumulate or to remain intact. This phenomenon of the slow elimination of radioactivity from blood cells was also found in the previous study. In HPLC, the plasma from treated rats showed the presence of unchanged pramipexole and 2-5 metabolites (data not shown). Consequently, the radioactivity distributed in blood cells may be due to metabolites, which are an N-despropyl metabolite and/or a hydroxy metabolite, and the increase in the blood cell/plasma ratio may reflect an increase in the ratio of metabolites. Most of the radioactive components in tissues at the late phase might also be metabolites.

In conclusion, it was observed in this study that the level of radioactivity in plasma would reach a steady state after 12 or 13 doses, and the elimination of radioactivity from plasma after the last dose would not be affected by repeated administration. Pramipexole would show the high levels in the liver and kidney but it would be eliminated from most tissues in parallel with plasma.

References


3) Piercey M. F.: Pharmacology of pramipexole, a dopamine D3-prefering agonist useful in treating Parkinson’s dis-

Table V Distribution of radioactivity into blood cells after repeated oral administration of 14C-pramipexole at a dose of 0.5 mg/kg once a day for 14 days to male rats

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Ht (%)</th>
<th>Concentration (ng eq./ml)</th>
<th>Blood</th>
<th>Plasma (a)</th>
<th>Blood cell (b)</th>
<th>b/a</th>
</tr>
</thead>
<tbody>
<tr>
<td>168</td>
<td>42.25±2.22</td>
<td>120.62±7.79</td>
<td>9.19±0.96</td>
<td>273.03±11.70</td>
<td>29.90±2.45</td>
<td></td>
</tr>
<tr>
<td>240</td>
<td>44.25±2.22</td>
<td>113.45±7.81</td>
<td>5.97±0.59</td>
<td>248.61±6.15</td>
<td>42.05±5.45</td>
<td></td>
</tr>
<tr>
<td>336</td>
<td>42.50±0.58</td>
<td>88.30±3.17</td>
<td>3.23±0.45</td>
<td>203.35±4.32</td>
<td>63.78±8.44</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as the mean±S.D. for four animals.

