III. 2 Synthesis and Biodistribution of $^{11}$C-labeled Pargyline, an Irreversible Inhibitor of Monoamine Oxidase

Ishiwata K., Ido T., Yanai K., Kawashima K., Miura Y., Monma M., Watanuki S., Takahashi T. and Iwata R.
Cyclotron and Radioisotope Center, Tohoku University

Several propynylamines are suicide inactivators of the monoamine oxidase (MAO), and the $^3$H- or $^{14}$C-labeled compounds were synthesized for the enzymatic studies.$^{1,2}$ These inhibitors labeled with positron emitter are potentially valuable for in vivo estimation of the concentration of MAO in experimental animal or human studies. We describe the synthesis of N-benzyl-N-[$^{11}$C]-methyl 2-propynylamine($^{11}$C-pargyline), a specific inhibitor for the type B MAO, by the reaction of N-demethylpargyline with $^{11}$CH$_3$I and its biodistribution in mice and rabbit.

\[
\begin{align*}
\text{N-Bz-CH$_2$NHCH$_2$C=CH} & \quad \xrightarrow{^{11}\text{CH}_3\text{I}} \quad \text{N-Bz-CH$_2$NCH$_2$C=CH} \\
\end{align*}
\]

Materials and Methods

Synthesis of $^{11}$C-pargyline: $^{11}$CH$_3$I was produced by using the automated synthesis system.$^{3,4}$ $^{11}$CH$_3$I was trapped in 1.5 ml acetone containing 5-10 mg N-benzyl 2-propynylamine(Aldrich, without purification) at dry-ice acetone temperature. The solution was heated at 50-60°C for 5-10 min. Unreached $^{11}$CH$_3$I was swept with a helium stream of 200 ml/min for 2-3 min at 50-60°C. Acetone was also removed with a helium stream. The residue was dissolved in 300 µl 60% MeOH and subjected to radio-high performance liquid chromatography on µBondapak C18 column(Waters, RCSS system). Elution was performed with 60% MeOH at flow rate of 2 ml/min(Fig. 1). The $^{11}$C-pargyline fraction was collected, acidified with 4 drops of 0.1 M HCl and evaporated to dryness. The $^{11}$C-pargyline was dissolved in saline, neutralized with 0.1 M NaOH and filtered through a membrane filter(0.22 µm). Radiochemical purity was analyzed by the above chromatographic system. Finally the $^{11}$C-pargyline was obtained for animal experiments with a radiochemical yield of 20%-45% and a radiochemical purity of over 99% for 50-60 min.

Biodistribution in mice: Mice were injected with non-carrier added $^{11}$C-pargyline intravenously. At various time intervals mice were killed by cervical dislocation, and tissues were removed and washed with saline. The tissues were weighed and the radioactivity was measured with an auto-well γ-counter. Data are expressed as the differential absorption ratio(DAR), (count/g tissue)x(g body weight/total injected count). The $^{11}$C-pargyline mixed with the
indicated amount of carrier pargyline hydrochloride (Sigma) was also injected into mice, and after 30 min the distribution was measured.

0.2-0.5 g of the brain, lung, kidney or liver was homogenized in 3 ml 0.3 M sucrose with a Teflon-glass homogenizer. The homogenate was separated into nuclear, crude mitochondrial and post mitochondrial fractions as described in previous report.\(^5,6\) The homogenate was also separated into the acid-soluble and acid-insoluble fractions.\(^5,6\)

Positron emission tomography in the rabbit heart and lung: 2 mCi \(^{11}\)C-pargyline was injected into a male rabbit intravenously. Emission scan was performed in the heart and lung region using the ECAT-II. The blood was collected at indicated time intervals and data are expressed as the DAR. The uptakes in the heart and lung were expressed as the DAR, \((\text{count/pixel}) \times (\text{g body weight/total injected count}).\)

Results

Table 1 shows the biodistribution of the \(^{11}\)C-pargyline in mice. The \(^{11}\)C-pargyline showed well transport into the many tissues and the rapid blood clearance. In the kidney the highest uptake except at 1 min after injection and slow clearance were observed. The liver uptake was similar but lower as the kidney uptake. In the lung the highest uptake just after injection, the rapid clearance for the first 10 min and very slow clearance thereafter were observed. In the brain and heart the \(^{11}\)C-pargyline was slowly cleared. In the pancreas, spleen and small intestine it was accumulated for the first 10 min and maintained. Generally, the tissue levels of the \(^{11}\)C-pargyline reached equilibrium after 30 min.

Table 2 shows the effect of the loading doses on the distribution of the \(^{11}\)C-pargyline. The liver, pancreas and spleen showed higher uptakes in the carrier added experiments than in the non-carrier added one. In the other tissues the uptakes tended to lower in the higher doses, but the differences were very small.

In the brain, lung, liver and kidney the subcellular distribution was examined (Table 3). Much \(^{11}\)C-radioactivity was observed in the nuclear and crude mitochondrial fractions. The proportion of the \(^{11}\)C in the two fractions in the brain were larger than those in other tissues. In the brain and liver, the proportions of the \(^{11}\)C in the crude mitochondrial fraction were larger than those in the nuclear fraction, but the reverse correlations were observed in the lung and kidney. The \(^{11}\)C-pargyline was incorporated into the acid-insoluble fraction with time, and its proportion reached to be 60%-70% of total radioactivity at 60 min. When the carrier pargyline (63 mg/kg) was injected simultaneously, the radioactivity of the nuclear and crude mitochondrial fractions were smaller than in the non-carrier added experiment.

Fig. 2A shows the positron emission tomogram of the \(^{11}\)C-pargyline in the heart and lung region of a rabbit. Fig. 2B shows the blood clearance and the heart and lung uptakes measured by the quantitative data collected in a time sequential study. Uptakes of the \(^{11}\)C-pargyline was kept in a constant level for
30 min in the heart. But in the lung it decreased gradually for the first 20 min and reached to a constant level. The blood clearance was rapid for the first 10 min.

Discussion

The $^{11}$C-pargyline was accumulated in many tissues and the levels of its concentration were constant after 30 min. Relative uptakes in the mouse heart and lung were different from those in rabbit. In the mice brain, lung, liver and kidney much $^{11}$C-radioactivity was distributed in the crude mitochondrial and nuclear fractions, and also incorporated into the acid-insoluble materials. Since the concentrations of MAO are different between tissues of the same spieces or animal spieces and MAO is firmly bound to the mitochondrial outer membrane and made irreversible complex with pargyline$^7)$, the levels of the $^{11}$C in the tissues may reflect the concentration of MAO. Relative uptakes of $^{11}$C-pargyline were similar to the relative concentration of type B MAO between the brain and lung in mice. However, the higher pargyline doses did not have much influence on the tissues uptakes, and the quantity of pargyline in crude mitochondrial fraction will be in excess of that of MAO$^8)$ although the proportion of the $^{11}$C in the crude mitochondrial fraction were lowered in the highest dose experiment. These results indicate that some $^{11}$C-pargyline is trapped in the tissues dependent on non-specific binding as indicated in vitro experiments.$^8)$ Therefore, the tissue uptakes of $^{11}$C-pargyline do not reflect directly the concentration of type B MAO, and some consideration being distinguishable between the specific uptake and the non-specific uptake will be required for estimating correctly the concentration of MAO in vivo-experiments.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research No. 58870064, Ministry of Education, Science and Culture, Japan. We are grateful Dr. K. Itoh for usefull advice and discussion in a positron emission tomographic study.

References

Table 1. Distribution of $^{11}$C-pargyline in mice.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Uptake (DAR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 min (n=2)</td>
</tr>
<tr>
<td>Blood</td>
<td>1.00 ± 0.04</td>
</tr>
<tr>
<td>Heart</td>
<td>1.06 ± 0.10</td>
</tr>
<tr>
<td>Lung</td>
<td>4.13 ± 0.89</td>
</tr>
<tr>
<td>Liver</td>
<td>1.26 ± 0.30</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.63 ± 0.01</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.32 ± 0.04</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.98 ± 0.09</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.39 ± 0.05</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.43 ± 0.07</td>
</tr>
<tr>
<td>Brain</td>
<td>1.01 ± 0.16</td>
</tr>
<tr>
<td>Testis</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>Bone</td>
<td>0.41 ± 0.35</td>
</tr>
</tbody>
</table>

Table 2. Effects of loading dose on distribution of $^{11}$C-pargyline in mice at 30 min after injection.

<table>
<thead>
<tr>
<th>Loading dose</th>
<th>non-carrier 0.5 mg/kg 5 mg/kg 50 mg/kg 50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=6</td>
<td>n=4</td>
</tr>
<tr>
<td></td>
<td>Uptake (DAR)</td>
</tr>
<tr>
<td>Blood</td>
<td>0.24 ± 0.05</td>
</tr>
<tr>
<td>Heart</td>
<td>0.52 ± 0.16</td>
</tr>
<tr>
<td>Lung</td>
<td>0.96 ± 0.34</td>
</tr>
<tr>
<td>Liver</td>
<td>1.03 ± 0.39</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.49 ± 0.72</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.88 ± 0.36</td>
</tr>
<tr>
<td>Small intestine</td>
<td>1.17 ± 0.50</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.83 ± 1.83</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.43 ± 0.16</td>
</tr>
<tr>
<td>Brain</td>
<td>0.34 ± 0.14</td>
</tr>
</tbody>
</table>
### Table 3. Subcellular distribution of $^{11}$C-pargyline in mouse tissues (n=2)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Fraction</th>
<th>10 min (%)</th>
<th>30 min (%)</th>
<th>30 min (+63mg/kg) (%)</th>
<th>60 min (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>Nuclear Fr.</td>
<td>11.3</td>
<td>23.2</td>
<td>13.3</td>
<td>14.3</td>
</tr>
<tr>
<td></td>
<td>Crude mitochondrial Fr.</td>
<td>34.2</td>
<td>37.9</td>
<td>23.7</td>
<td>32.5</td>
</tr>
<tr>
<td></td>
<td>Post mitochondrial Fr.</td>
<td>56.3</td>
<td>38.9</td>
<td>63.0</td>
<td>53.3</td>
</tr>
<tr>
<td></td>
<td>Acid-insoluble Fr.</td>
<td>38.2</td>
<td>46.1</td>
<td></td>
<td>58.6</td>
</tr>
<tr>
<td>Lung</td>
<td>Nuclear Fr.</td>
<td>16.5</td>
<td>28.2</td>
<td>16.6</td>
<td>25.3</td>
</tr>
<tr>
<td></td>
<td>Crude mitochondrial Fr.</td>
<td>9.3</td>
<td>13.3</td>
<td>7.8</td>
<td>12.1</td>
</tr>
<tr>
<td></td>
<td>Post mitochondrial Fr.</td>
<td>74.2</td>
<td>58.5</td>
<td>80.6</td>
<td>62.6</td>
</tr>
<tr>
<td></td>
<td>Acid-insoluble Fr.</td>
<td>41.3</td>
<td>56.8</td>
<td></td>
<td>58.6</td>
</tr>
<tr>
<td>Liver</td>
<td>Nuclear Fr.</td>
<td>5.0</td>
<td>13.7</td>
<td>17.1</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>Crude mitochondrial Fr.</td>
<td>15.4</td>
<td>21.3</td>
<td>11.2</td>
<td>19.1</td>
</tr>
<tr>
<td></td>
<td>Post mitochondrial Fr.</td>
<td>79.6</td>
<td>65.1</td>
<td>71.7</td>
<td>73.4</td>
</tr>
<tr>
<td></td>
<td>Acid-insoluble Fr.</td>
<td>38.7</td>
<td>46.8</td>
<td></td>
<td>67.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>Nuclear Fr.</td>
<td>14.5</td>
<td>15.1</td>
<td>10.6</td>
<td>32.9</td>
</tr>
<tr>
<td></td>
<td>Crude mitochondrial Fr.</td>
<td>7.0</td>
<td>9.6</td>
<td>6.7</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td>Post mitochondrial Fr.</td>
<td>78.5</td>
<td>75.3</td>
<td>87.2</td>
<td>58.2</td>
</tr>
<tr>
<td></td>
<td>Acid-insoluble Fr.</td>
<td>36.1</td>
<td>37.8</td>
<td></td>
<td>69.8</td>
</tr>
</tbody>
</table>

**Fig. 1.** Separation of $^{11}$C-pargyline on radio-high performance liquid chromatography. Details are described in Materials and Methods. The dotted line shows $^{11}$C-radioactivity response. Arrow shows the elution position of an authentic pargyline.
Fig. 2. Distribution of $^{11}$C-pargyline in the rabbit. A) Positron emission image in the rabbit heart and lung region at 25-30 min after injection. B) Changes in the $^{11}$C concentration in the heart, lung and blood. Concentrations in the heart and lung were calculated in the areas shown H and L, respectively, in Fig. 2A.