III. 2 Biological Evaluation of [5-$^{11}$C-methoxy]Donepezil in the Rat Brain


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Introduction

Alzheimer’s disease (AD) is an age-related and progressive neurodegenerative disease of the central nervous system. At the cellular levels, there were marked neurodegenerative changes accompanied with a reduction in many neurotransmitters and neuropeptides. A remarkable dysfunction of the cholinergic system, characterized by deficits in memory and cognitive functions, has been observed in several brain regions of patients suffering from AD$^{1,2,3}$ Especially, acetylcholinesterase (AChE, EC 3.1.1.7) activities in the neocortex and the hippocampus is reported to be lowered in AD$^4$, and decreased activity correlates with the severity of cognitive impairment$^5$. These pathological findings have led to the hypothesis that enhancement cholinergic neurotransmission with cholinergic agent, either AChE inhibitors or cholinergic agonists, may ameliorate the cognitive impairment in AD. Although many attempts have been made to reverse cognitive impairment using cholinergic agents, AChE inhibitors are the only class of drugs for the treatment of AD. Recently, one promising AChE inhibitor, donepezil, was developed and has been successfully used for treatment of AD. It is reported that this agent has a high affinity and selectivity for AChE with an excellent efficacy and fewer undesired pharmacological effects$^6$.

In the present study, we describe the synthesis of [5-$^{11}$C-methoxy]donepezil using $^{[11}C]$methyl triflate from 5’-$O$-desmethylprecursor, and the biological evaluation of [5-$^{11}$C-methoxy]donepezil.
Materials and Methods

Male Wistar rats (Japan SLC, Shizuoka, Japan; 5 weeks) were used in these studies. They were fed food and water ad libitum. The animal study was carried out according to the protocol approved by the Animal Care Committees of Cyclotron and Radioisotope Center.

The rats were injected intravenously with 7.4 MBq (200 µCi) of \(^{11}\text{C}\)donepezil in 0.2 mL of physiological saline via tail vein. The rats were killed at the four time points (10, 20, 40 and 60 min). Samples of blood and six brain tissues (cerebral cortex, striatum, hippocampus, cerebellum, midbrain and brain stem) were quickly removed, weighed, and counted. The amount of radioactivity was expressed as DAR (differential absorption ratio). DAR is defined as follows; [observed radioactivity in the tissue] x [body weight] / [weight of tissue] / [injected radioactivity].

The saturation experiments were performed in order to demonstrate the reversibility of \(^{11}\text{C}\)donepezil binding in vivo. A large amount of unlabeled donepezil (0.1, 1 or 5 mg/kg) was intraperitoneally (i.p) administered with rats 20 min before \(^{11}\text{C}\)donepezil injection. The rats were sacrificed 40 min after the injection.

Results and Discussion

The radio-synthesis was carried out by the loop-SPE method described in our previous paper\(^7,8,9\). \(^{11}\text{C}\)Donepezil was successfully synthesized by \(O\)-methylation of 5’-\(O\)-desmethylprecursor (M2) using the loop method with \(^{11}\text{C}\)MeOTf (Fig.1). The radioactivity of \(^{11}\text{C}\)donepezil was approximately 92.5-814 MBq (2.5-22 mCi) after 20 min irradiation and radiochemical yield was calculated to be 25-30% based on \(^{11}\text{C}\)MeOTf after decay-correction. The specific activity of \(^{11}\text{C}\)donepezil was 19-122 GBq/µmol (0.51-3.30 Ci/µmol) at the end of synthesis (30-40 min after the bombardment). The radiochemical purity was more than 99%.

Table 1 demonstrates the time course of \(^{11}\text{C}\)donepezil binding in vivo. The values of DAR in brain tissues were higher than that in the blood, suggesting that \(^{11}\text{C}\)donepezil easily penetrates through the blood-brain barrier. Forty min after injection, the distribution of \(^{11}\text{C}\)donepezil was heterogeneous in the brain; it was especially higher in the striatum and brain stem. Our in vitro binding study demonstrated \(^{11}\text{C}\)donepezil binding was the lowest in the cortex\(^9\). Therefore, the data of in vivo binding was calculated as the ratio of tissue to cortex. The ratios of striatum-to-cortex and brain stem-to-cortex were approximately 1.4 and 1.5 respectively.

In the blocking study, the distribution was measured 40 min after \(^{11}\text{C}\)donepezil
injection. The ratios of striatum-to-cortex and brain stem-to-cortex were significantly reduced from $1.4 \pm 0.2$ to $1.0 \pm 0.1$ $(n=4, p<0.05)$ and $1.5 \pm 0.1$ to $1.1 \pm 0.1$ $(n=4, p<0.01)$, respectively, by the administration of donepezil (5 mg/kg, i.p.). To confirm the reversibility of $[^{11}\text{C}]$donepezil binding in vivo, the dose effects on tissue to cortex ratios were examined 40 min after the injection of $[^{11}\text{C}]$donepezil. As shown in Fig.2, the striatum-to-cortex and the brain stem-to-cortex ratios were reduced with increasing amounts of donepezil, whereas the hippocampus-to-cortex ratio did not change with the injected dose. This result indicates that $[^{11}\text{C}]$donepezil specifically binds to the AChE rich regions. On the other hand, the binding in the hippocampus was not displaced by unlabeled donepezil, indicating the higher nonspecific binding of $[^{11}\text{C}]$donepezil to the hippocampus.

In conclusion, we have successfully synthesized $[5-^{11}\text{C}-\text{methoxy}]$donepezil using $[^{11}\text{C}]$MeOTf. In vivo brain distribution of $[^{11}\text{C}]$donepezil in the rat brain was heterogeneous, and it was displaced by unlabeled donepezil. Although further studies are needed, this study clearly demonstrates that this strategy is useful to visualize AChE in the living human brain and to evaluate the efficacy of therapy of AChE inhibitors.

References

Table 1. *In vivo* distribution of $[^{11}C]$donepezil in the rat blood and brain.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>10 min</th>
<th>20 min</th>
<th>40 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.23 ± 0.02</td>
<td>0.17 ± 0.01</td>
<td>0.10 ± 0.00</td>
<td>0.13 ± 0.00</td>
</tr>
<tr>
<td>Cortex</td>
<td>0.48 ± 0.09</td>
<td>0.53 ± 0.02</td>
<td>0.57 ± 0.08</td>
<td>0.29 ± 0.01</td>
</tr>
<tr>
<td>Striatum</td>
<td>0.74 ± 0.09</td>
<td>0.61 ± 0.06</td>
<td>0.72 ± 0.08</td>
<td>0.37 ± 0.03</td>
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<tr>
<td>Hippocampus</td>
<td>0.41 ± 0.06</td>
<td>0.49 ± 0.01</td>
<td>0.58 ± 0.07</td>
<td>0.33 ± 0.06</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.49 ± 0.07</td>
<td>0.56 ± 0.02</td>
<td>0.64 ± 0.07</td>
<td>0.38 ± 0.01</td>
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<tr>
<td>Midbrain</td>
<td>0.47 ± 0.07</td>
<td>0.56 ± 0.02</td>
<td>0.65 ± 0.07</td>
<td>0.36 ± 0.01</td>
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<tr>
<td>Brain stem</td>
<td>0.53 ± 0.04</td>
<td>0.65 ± 0.03</td>
<td>0.85 ± 0.14</td>
<td>0.55 ± 0.02</td>
</tr>
</tbody>
</table>

DAR values are expressed as means ± S.E.M (n=3–8)

Fig. 1. Radio-synthesis of $[^{11}C]$donepezil.

Fig. 2. The *in vivo* binding of $[^{11}C]$donepezil. Dose-dependent inhibition of $[^{11}C]$donepezil binding *in vivo*. Tissue-to-cortex ratios as a function of donepezil dose at 40 min after $[^{11}C]$donepezil injection.