VII. 1. Synthesis and Preclinical Evaluation of a Fluorine-18 Labeled BF-227 Derivative

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Introduction

Alzheimer's disease (AD) is an age-dependent and irreversible neurodegenerative disorder leading to deterioration of memory and cognitive functions and the most common form of senile dementia. Although the exact mechanisms underlying pathogenesis of AD are not fully understood, formation of brain amyloid plaques through aggregation and deposition of β-amyloid proteins (Aβ) is considered to be the initial pathogenic event which precedes the appearance of clinical AD symptoms by decades. Thus, a non-invasive approach for detecting the amyloid plaques in the living brain could hold considerable promise for more accurate antemortem diagnosis of AD in the early and perhaps pre-symptomatic phases of AD. For that purpose, many PET tracers have been reported to date, such as ^{11}C-PiB, ^{11}C-SB-13, ^{18}F-florbetapir, ^{18}F-flutemetamol, ^{18}F-florbetaben and so on[1)]. We also had reported that ^{11}C-BF-227 is a promising PET ligand for in vivo detection of dense amyloid deposits in AD patients. Considering accessibility of the ligand, development of a new BF-227 derivative labeled with fluorine-18, which has a longer half-life than carbone-11, would be valuable. In this study, we synthesized several kinds of ^{18}F-labeled analogues of BF-227 and examined their feasibility for in vivo amyloid imaging[2)].

Methods

Fluorine-18 labeled derivatives of BF-227 were newly designed and synthesized as indicated in Fig. 1. The fluoroethoxy and dimethylamino groups of BF-227 were modified to optimize the probe properties for amyloid imaging. To confirm binding of these

compounds to amyloid plaques, fluorescent staining of AD brain sections with them. Biodistribution study using the ^{18}F-labeled derivatives and normal ICR mice was conducted to assess their brain kinetics and in vivo defluorination. Through these experiments, we selected ^{18}F-THK-763 as a potent agent for amyloid imaging. Binding nature of ^{18}F-THK-763 to Aβ plaques was evaluated by in vitro binding assay using Aβ fibrils, in vitro autoradiography (ARG) of AD brain sections, and ex vivo ARG of the brain of APP23 mouse and wild type one. Additionally, small animal PET imaging was performed using APP23 in comparison to ^{11}C-PiB. Finally, ability of of ^{18}F-THK-763 (^{18}F-FACT) to image amyloid depositions was preliminarily evaluated through human PET imaging study in AD patients.

Results and Discussion

Fluorescent staining of the AD brain sections revealed that senile plaques were clearly stained by the fluorinated BF-227 derivatives. Biodistribution study of the ^{18}F-labeled derivatives in normal mice elucidated that ^{18}F-THK-763 possesses the most favorable in vivo properties; rapid brain uptake (4.64 %ID/g at 2min), fast clearance from the normal brain without non-specific binding (0.28 %ID/g at 60min), and resistance to metabolic defluorination (low radioactivity uptake in bone) (Fig. 2). In addition, in vitro binding assay showed that ^{18}F-THK-763 binds to Aβ fibrils with a high binding affinity (Kd: 3.6 nM). ARG study using AD brain sections indicated a specific binding of ^{18}F-THK-763 to the cerebral amyloid plaques. Ex vivo ARG of the brain of APP23 mouse (Fig. 3A) and wild type mouse (Fig. 3B) using ^{18}F-THK-763 also demonstrated that the radioactive spots (Fig. 3C) were observed only in the APP-mouse brain and the distribution was identical (Fig. 3D) to that of amyloid plaques labeled with Thioflavin-S (Fig. 3E). PET images of wild-type and APP transgenic mice indicated that amyloid deposits in the cerebral cortices of APP23 mice were clearly visualized with the same distribution pattern between ^{18}F-THK-763 and ^{11}C-PiB. PET imaging in the normal aged (NA) subject and AD patient, still preliminary, showed significantly higher ^{18}F-THK-763 uptake in the neocortex compared to NA subjects. Significant correlation between regional SUVR of ^{18}F-THK-763 and ^{11}C-BF-227 were observed in 2 AD patients and one NA subject.

Conclusions

In conclusions, we had succeeded in developing a new ^{18}F-labeled amyloid

tracer, ^{18}F-THK-763. It showed a rapid and an adequate brain uptake, smooth washout from the normal mouse brain, no metabolical defluorination in vivo, a high binding affinity to Aβ fibrils and plaques, and a high retention in the brain regions containing amyloid plaques in both APP23 mice and AD patients by PET. These results strongly suggest that ^{18}F-THK-763 has enough potential for in vivo imaging of amyloid deposits in the AD brain.

References

1) Furumoto S, Okamura N, Iwata R, Yanai K, Arai H, Kudo Y., *Curr. Top. Med. Chem.* **7** (2007) 1773.
2) Furumoto S, Okamura N, Furukawa K, et al., *Mol. Imaging Biol.* **15** (2013) 497.

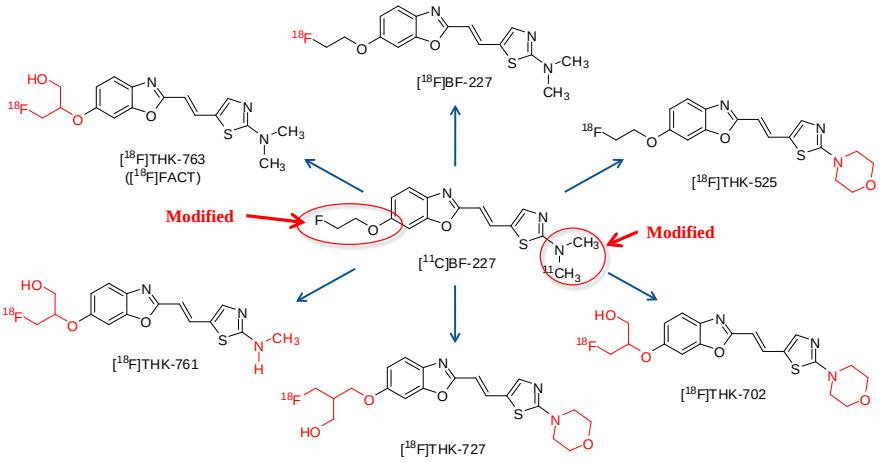

Figure 1. Fluorine-18 labeled BF-227 derivatives synthesized and evaluated in this study.

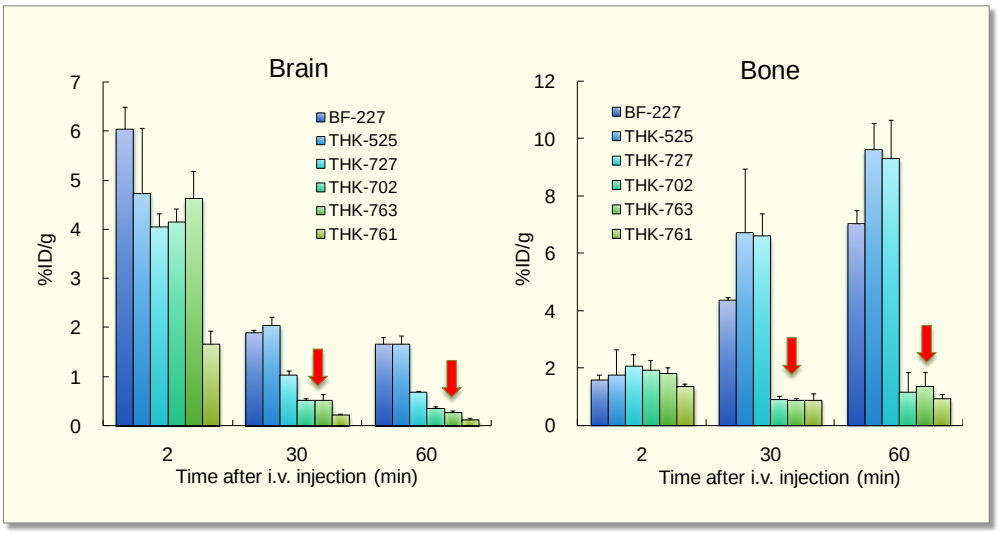

Figure 2. Radioactivity uptakes in the brain and bone of normal mice (n=4) after i.v. injection of the ^{18}F-labeled derivatives.

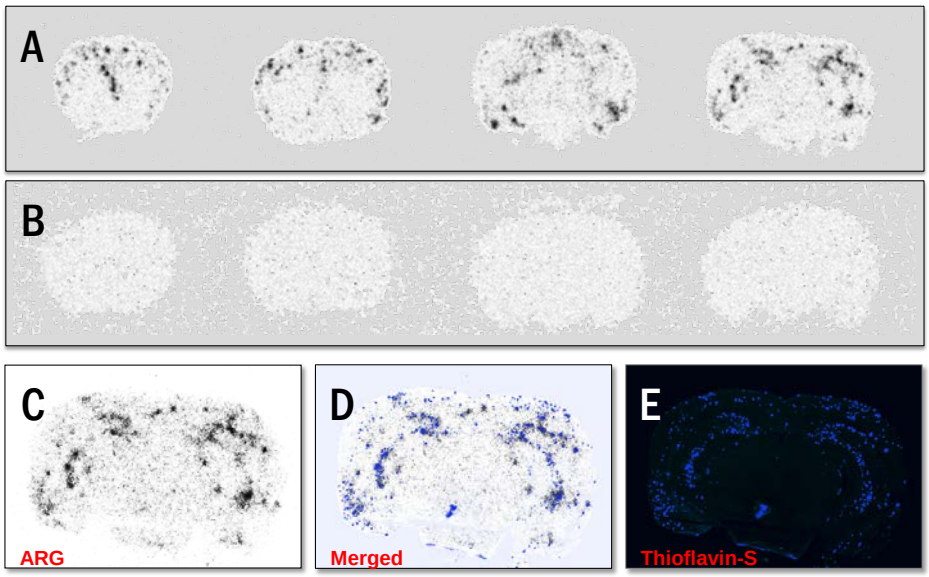

Figure 3. Ex vivo ARG of the brain of APP23 mouse and wild type one.