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論文題目	Development of Benzofuran Derivatives as Molecular Probes for in vivo Imaging of β-Amyloid Plaques in Alzheimer's Disease Brains (アルツハイマー病脳内 β アミロイドプラークの生体イメージング用分子プローブとしてのベンゾフラン誘導体の開発)		

（論文内容の要旨）

Alzheimer's disease (AD) is an age-related brain disorder with the symptoms of memory loss and dementia. Since postmortem brains of AD patients show the presence of senile plaques containing β-amyloid peptides (Aβ) as a neuropathological feature and the formation of β-amyloid plaques is considered to be an initial neurodegenerative event in AD brains, quantitative evaluation of β-amyloid plaques in the brain with noninvasive techniques, such as positron emission tomography (PET) and single photon emission computed tomography (SPECT), would allow a presymptomatic diagnosis and the monitoring of putative effects of neuroprotective treatments. In the present study, I planned to develop novel PET and SPECT imaging probes based on benzofuran scaffold for detection of β-amyloid plaques in AD brains.

Chapter 1: Development of PET imaging probes for in vivo imaging of β-amyloid plaques in Alzheimer's disease brains.

To define an acceptable imaging probe for β-amyloid plaques in vivo, some common criteria are applied, such as 1) a sufficient amount of the agent to enter the brain; 2) the agent should have sufficient affinity for Aβ; 3) the non-specifically bound agent should be cleared from the brain. From the viewpoint of the criteria, based on the structure of the amyloid-staining dye, thioflavin T, a novel series of fluorinated benzofuran derivatives were synthesized and evaluated as potential tracers for PET targeting β-amyloid plaques in the brains of patients with AD. The formation of benzofuran scaffold was carried out using Suzuki coupling reaction. In experiments in vitro, all derivatives displayed high affinity for Aβ(1-42) aggregates with K_i values in the nanomolar range. In biodistribution experiments using normal mice, ^{18}F-labeled benzofuran derivatives displayed good initial uptake in the brain. A pyridyl benzofuran derivative [^{18}F]FPYBF-2, in particular, showed a high initial uptake and a reasonable washout from the brain, which is highly desirable for β-amyloid imaging probes. In experiments in vivo, [^{18}F]FPYBF-2 showed extensive labeling of β-amyloid plaques in the Tg2576 transgenic mice, which are specifically engineered to overproduce β-amyloid plaques in the brain, but not in the age-matched controls. Furthermore, the specific labeling of β-amyloid plaques by [^{18}F]FPYBF-2 was observed in autoradiographs of sections of autopsied AD brain. The results suggest that [^{18}F]FPYBF-2 may be a promising PET probe for imaging cerebral β-amyloid plaques.

Chapter 2: Development of SPECT imaging probes for in vivo imaging of β-amyloid plaques in Alzheimer's disease brains.

In consideration of routine clinical use such as longer half-lives and lower costs, based on the positive results in chapter 1, ^{123}I- and ^{99m}Tc-labeled tracers with the backbone of pyridyl benzofuran were designed and tested for imaging β-amyloid plaques using SPECT. In this study, ^{125}I was used instead of ^{123}I for experimental convenience.

Firstly, I had planed that ^{123}I, which is the congener of ^{18}F and also a radionuclide for SPECT imaging, is introduced into the backbone of pyridyl benzofuran. Similar to fluorinated pyridyl

benzofuran derivatives, the iodinated pyridyl benzofuran derivatives also displayed affinity for Aβ(1-42) aggregates and ^{125}I-labeled compounds showed specific labeling of β-amyloid plaques in sections of autopsied AD brain. In biodistribution experiments using normal mice, Labeled compounds with ^{125}I showed good initial uptake in the brain and fast washout from the brain, which is more favorable for in vivo imaging of β-amyloid plaques than ^{18}F-labeled pyridyl benzofuran derivatives. The preliminary study showed promise for imaging cerebral β-amyloid plaques with SPECT.

Among medical radioisotopes, ^{99m}Tc ($T_{1/2}$ = 6.01 h, 141 keV) has been most commonly used in nuclear medical diagnostic field because of favorable radiophysical characteristics for in vivo imaging by SPECT. Thus, ^{99m}Tc-labeled pyridyl benzofuran derivatives were designed as potential probes for imaging β-amyloid plaques. Different from ^{18}F and ^{123}I, a chelating structure is necessary for the transition of metal ^{99m}Tc to an organic molecule. In consideration of the permeability of the blood-brain barrier, bisaminoethanethiol (BAT) was chosen as a chelating ligand to form a neutral and compact complex with ^{99m}Tc. As there is no stable technetium isotope, rhenium, the congener of technetium, has been widely used as a non-radioactive surrogate for the structural identification of technetium complexes. Then, I synthesized the Re complexes. In experiments in vitro, all the Re complexes showed high affinity for Aβ(1-42) aggregates. Biodistribution experiments in normal mice revealed that the ^{99m}Tc-labeled derivatives displayed sufficient uptake in the brain. Among the compounds tested, [^{99m}Tc]BAT-Bp-2 displayed substantial labeling of β-amyloid plaques in the ex vivo autoradiography in sections of brain tissue from Tg2576 transgenic mice but not in the age-matched controls, indicating that [^{99m}Tc]BAT-Bp-2 shows specific binding to β-amyloid plaques in vivo. Gathered data demonstrated that [^{99m}Tc]BAT-Bp-2 may be a potential SPECT probe for imaging β-amyloid plaques in the brain with Alzheimer's patients.

In conclusion, I designed, synthesized and tested a novel series of benzofuran derivatives as potential PET and SPECT imaging probes for β-amyloid plaques in the brain. Among them, I successfully developed [^{18}F]FPYBF-2 and [^{99m}Tc]BAT-Bp-2 which not only displayed the high initial uptake into and reasonable washout from the brain, but also showed extensive labeling of β-amyloid plaques in vivo. Further investigation in living animals and humans with these imaging probes developed will provide useful information on the development of diagnostic method for AD and the drug development for treatment of AD in the future.

（続紙 ２ ）

（論文審査の結果の要旨）

脳内の β アミロイドプラークの蓄積はアルツハイマー病の代表的な病理学的特徴であることから、PET、SPECT を用いる核医学的手法による脳内 β アミロイドプラークの生体イメージングはアルツハイマー病の診断、治療薬開発などの観点から期待されている。そこで本論文では、脳 β アミロイドプラークの生体イメージングを目的とした PET および SPECT 用分子プローブの開発を計画したものである。

著者は、先ず、脳への高い移行性、βアミロイドプラークへの結合性、βアミロイドプラークに結合していない場合の脳からの速やかな消失を条件として、現在βアミロイドプラークのインビトロでの蛍光染色試薬として利用されているチオフラビンTをリード化合物として、PET用核種^{18}Fで標識された種々のピリジルベンゾフラン誘導体を設計、合成し、その中で [^{18}F]FPYBF-2 が最も有効な性質を有することを見出した。さらに、アミロイド前駆タンパク質を過剰発現させたトランスジェニックマウス(Tg2576 マウス)に投与してPETイメージングを行った結果、野生型マウスに比べて高い脳への放射能集積とその脳切片のオートラジオグラムでのβアミロイドプラークに一致した放射能集積を認めた。また、アルツハイマー病脳切片を用いた実験でも同様の結果を得、PET用βアミロイドプラークイメージングプローブとしての [^{18}F]FPYBF-2 の開発に成功した。

上記の結果を基盤に、さらにSPECT用βアミロイドプラークイメージングプローブの開発を計画し、^{123}Iで標識されたピリジルベンゾフラン誘導体の開発にも成功した。さらに、^{99m}Tc標識プローブの開発も試み、^{99m}Tc を安定に結合させるためにはキレート形成部位が必須であることから、bisaminoethanethiol (BAT) をキレート形成部位として選択し、それを有する[^{99m}Tc]BAT-Bp-2 を合成した。本化合物は、Tg2576 マウスにおいて、脳への移行性とその脳切片オートラジオグラムでのβアミロイドプラークに一致した放射能集積を認め、^{99m}Tc標識化合物として、静脈投与後に脳内βアミロイドプラークに結合する化合物を開発することに初めて成功した。

以上、本研究は、脳内の β アミロイドプラークの生体イメージングのための PET および SPECT 用分子プローブの開発に成功したものであり、これらの知見は、今後のアルツハイマー病の画像診断、さらには治療薬開発に有益な情報を提供するものと評価される。

よって本論文は博士（薬学）の学位論文として価値あるものと認める。

さらに、平成２４年２月２７ 日論文内容とそれに関連した口頭試問を行った結果、合格と認めた。

論文内容の要旨及び審査の結果の要旨は、本学学術情報リポジトリに掲載し、公表とする。特許申請、雑誌掲載等の関係により、学位授与後即日公表することに支障がある場合は、以下に公表可能とする日付を記入すること。

要旨公開可能日： 平成　　年　　月　　日以降