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J Neurol (2010) 257:721–727
DOI 10.1007/s00415-009-5396-8

ORIGINAL COMMUNICATION

Amyloid PET in mild cognitive impairment and Alzheimer's disease with BF-227: comparison to FDG–PET

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Received: 6 June 2009 / Revised: 29 August 2009 / Accepted: 10 November 2009 / Published online: 28 November 2009

Abstract We recently developed a novel PET tracer, ^{11}C-labeled 2-(2-[2-dimethylaminothiazol-5-yl]ethenyl)-6-(2-[fluoro]ethoxy)benzoxazole ([^{11}C]BF-227), and had success with in vivo detection of amyloid plaques in Alzheimer's disease (AD) brains (Kudo et al. in J Nucl Med 8:553–561, 2007). We applied this tracer to subjects with mild cognitive impairment (MCI) and AD in order to elucidate the status of amyloid plaque deposition in MCI and compared the diagnostic performance of BF-227-PET with that of FDG–PET in AD cases. We studied 12 aged normal (AN) subjects, 15 MCIs and 15 ADs with PET using [^{11}C]BF-227. PET images were obtained after administration of BF-227 and the regional standardized uptake value (SUV) and the ratio of regional to cerebellar SUV were calculated as an index of BF-227 binding. AD patients showed increased uptake of [^{11}C]BF-227 in the neocortical areas and striatum as well as decreased glucose metabolism in temporoparietal, posterior cingulate and medial temporal areas. MCI subjects showed a significant increase in BF-227 uptake in the neocortical areas similar to AD, and the most significant difference of BF-227 retention was observed in the parietal lobe if its retentions for MCI were compared to those for AD and AN. On the other hand, glucose hypometabolism in MCI was confined to cingulate and medial temporal cortices. Neocortical BF-227 uptake negatively correlated with glucose metabolism. Receiver operating characteristic (ROC) analysis indicated higher specificity and sensitivity with BF-227-PET than those with FDG–PET for differential diagnosis between AD and normal control. We conclude that [^{11}C]BF-227-PET has a possibility to be a useful technology for early detection of AD pathology and also even in the MCI stage.

Keywords Alzheimer's disease · Amyloid · Senile plaque · PET · MCI

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Introduction

Senile or amyloid plaque is a pathological hallmark of Alzheimer's disease (AD), and amyloid β peptide (Aβ), which is a main component of the senile plaque, is believed to play a key role in the pathogenesis of AD [8]. In recent years several laboratories, including ours, have succeeded in visualizing Aβ deposition in living patients' brains with

AD using PET probes [13, 14, 24]. Pittsburgh Compound-B (PIB), which is the most commonly used probe for Aβ now, has been applied not only to AD but also to several other neurological disorders [15, 24].

Petersen from the Mayo clinic addressed the concept of mild cognitive impairment (MCI), which is an intermediate state between normal aging and AD [20, 21]. The criteria he stated for MCI are cognitive concern expressed by a physician, informant, participant or nurse; cognitive impairment in one or multiple domains (executive function, memory, language or visuospatial); normal functional activities; not demented.

Regional cerebral glucose metabolism (rCMRglu) has been studied by several investigators [9, 18, 19] using [^{18}F] 2-fluoro-deoxy-D-glucose (FDG) and PET in diseases causing dementia including AD. We used BF-227-PET as well as FDG–PET on the same subjects (AN, MCI, and AD) and carefully analyzed and compared the results with these two kinds of PET. Finally using these data we investigated and compared the specificity and sensitivity of BF-227 PET and FDG–PET in diagnosing AD.

Method

Twelve ANs, 15 subjects with MCI and 15 patients with AD were recruited in the present study. The demographic information of the subjects is shown in Table 1. The diagnosis for MCI and probable AD followed the MCI clinical criteria presented by "Petersen et al." [20] and "the National Institute of Neurological and Communicative Disorders and Stroke—Alzheimer's Disease and Related Disorders Association" [17], respectively. In 15 MCI subjects, 10 were amnestic multi-domain MCI and the other 5 subjects were amnestic single-domain MCI. Mini-mental state examination (MMSE) scores were significantly different between "AN and MCI", "AN and AD", and "MCI and AD". The study protocol was approved by the Committee on Clinical Investigation at Tohoku University School of Medicine and the Advisory Committee on Radioactive Substances at Tohoku University. After a complete description of the study to the patients and subjects, written informed consent was obtained.

Table 1 Demographic details of the subjects in this study

	N	Gender	Age	MMSE
AN	12	M/F = 7/5	66.3 ± 3.3	29.9 ± 0.3
MCI	15	M/F = 8/7	78.3 ± 3.8	25.5 ± 2.5
AD	15	M/F = 5/10	72.5 ± 6.9	19.5 ± 3.7

AN aged normal, *MCI* mild cognitive impairment, *AD* Alzheimer's disease. MMSE scores are significantly different between "AN and MCI", "AN and AD", and "MCI and AD"

The PET procedure for BF-227 was described precisely before [14]. BF-227 and its *N*-desmethylated derivative (a precursor of [^{11}C]BF-227) were custom-synthesized by Tanabe R&D Service Co. [^{11}C]BF-227 was synthesized from the precursor by *N*-methylation in dimethyl sulfoxide using [^{11}C]methyl triflate. The [^{11}C]BF-227 PET study was performed using a PET SET-2400 W scanner (Shimadzu Inc., Japan). After intravenous injection of 211–366 mBq of [^{11}C]BF-227, dynamic PET images were obtained for 60 min with each subject's eyes closed. Standardized uptake value (SUV) images of [^{11}C]BF-227 were obtained by normalizing tissue radioactivity concentration by injected dose and body weight. The FDG–PET procedure was described previously [19]. Subjects were scanned in a quiet and dimly-lit room with their eyes closed after at least 4 h of food restriction. Following a 68 Ga/Ga transmission scan of 7 min duration, an emission scan, which lasted 60 min after intravenous injection of FDG, was performed. The emission data were corrected for tissue attenuation using the transmission data. Regions of interest (ROIs) were placed on individual axial magnetic resonance (MR) images in the cerebellar hemisphere, striatum, frontal, lateral temporal, medial temporal, parietal, occipital, anterior and posterior cingulate cortices. The ROI information was then copied onto dynamic PET SUV images, and regional SUVs were sampled using Dr. View/LINUX software (AJS inc., Japan). Because there were neither senile plaques nor glucose hypometabolism in the cerebellum of AD, ratios of regional SUV to cerebellar SUV (SUVR) were calculated as an index of [^{11}C]BF-227 retention and CMRglu. Neocortical SUVR was calculated by averaging SUVRs in the frontal, lateral temporal, parietal and posterior cingulate cortices.

For statistical comparison in the three groups, we applied one-way analysis of variance (ANOVA) followed by the Bonferroni-Dunn post hoc test. The performance of diagnostic indices to discriminate among groups was assessed using the ROC analysis. Areas under ROC curves (AUC) were calculated and compared using GraphPad Prism Software (GraphPad Software Inc., San Diego, CA). Statistical significance was defined as $p < 0.05$.

Results

BF-227 retention in MCI

First, we analyzed PET images with [^{11}C]BF-227 among the three groups (AN, MCI, and AD), and representative brain PET images are shown in Fig. 1. As indicated in the figure, some MCI subjects showed strong retention of [^{11}C]BF-227, but other MCI subjects did not. Most AD cases, however, indicated strong accumulation of [^{11}C]BF-227 especially in

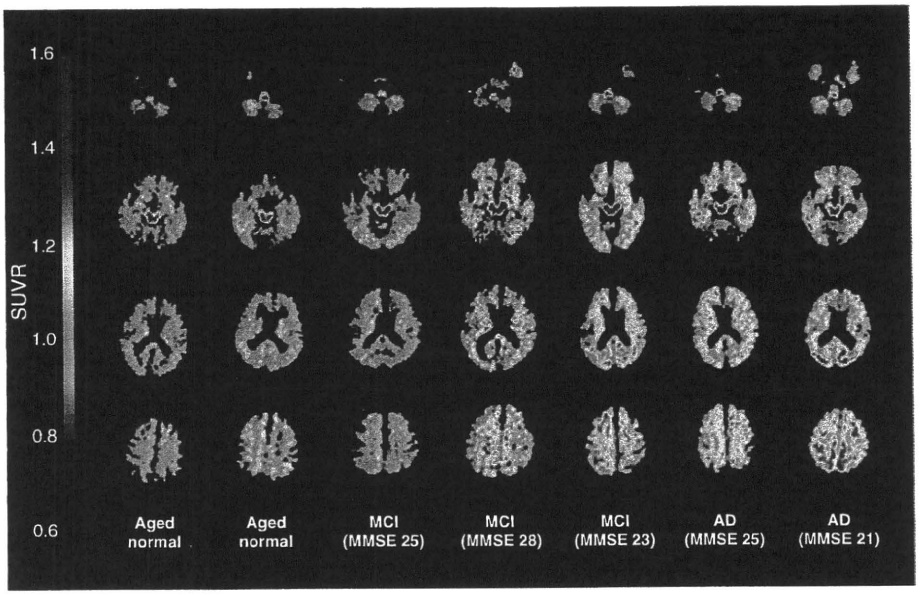

Fig. 1 Representative axial brain PET images with BF-227. Both the AD cases showed high SUVR compared to the aged normal subjects, although the MCI cases showed heterogeneity, that is, one MCI case (MMSE = 25) showed a comparative SUVR level to AN but another case showed SUVR as high as the AD level

frontal, temporal and parietal cortices. If the retention pattern of [^{11}C]BF-227 is compared to that of PIB, the accumulation of [^{11}C]BF-227 in the frontal lobe looks much weaker than that of PIB [3].

Figure 2 shows the mean neocortical and regional SUVRs of [^{11}C]BF-227 for the three groups. Both the mean neocortical SUVRs for MCI and AD are significantly higher than that for AN. As we previously reported [1], significantly higher SUVRs were observed in most cerebral regions in AD compared to AN except for the medial temporal lobe. MCI subjects indicated a significantly increased SUVR in frontal, lateral temporal, parietal, occipital cortices as well as anterior cingulate gyrus compared to AN, and the most prominent increase was observed in the lateral temporal cortex. A significantly lower SUVR in MCI was observed in the parietal cortex compared to AD. In the other neocortical regions, MCI subjects showed a tendency towards milder retention of BF-227 than that in AD. In the relationship between BF retentions and MMSE scores in all the subjects together (NC, MCI, and AD), no strong correlations were observed (data not shown).

Cerebral glucose metabolism in AN, MCI and AD

Next, we analyzed CMRglu in the same subjects using FDG–PET in order to compare to the findings with [^{11}C]BF-227, which is considered to indicate amyloid plaque depositions. As a result, a significant reduction of neocortical SUVR was observed in both MCI and AD patients compared to AN in FDG–PET (Table 1; Fig. 3). Regional SUVR in FDG–PET was significantly decreased in the cingulate gyrus and medial temporal cortex of MCI subjects and in the lateral temporal, parietal, posterior cingulate and medial temporal cortices of AD patients, compared to AN. Table 2.

Neocortical SUVR of FDG–PET for each subject was plotted against neocortical SUVR of BF-227-PET (Fig. 4a). SUVR of BF-227 negatively correlated to SUVR of FDG in analyzing the subjects from three groups all together ($r = -0.337$, $p = 0.029$). A significant correlation of regional SUVR in BF-227-PET and FDG–PET was also observed in the temporal and parietal cortices (data not shown). However, no significant correlation was observed when the analysis was confined to the subjects in each group.

Furthermore, in order to compare sensitivity and specificity to differentiate AD from AN, ROC analysis was performed for the lateral temporal SUVR of BF-227 and posterior cingulate SUVR of FDG (Fig. 4b). The AUC for BF-227 (0.994) is much higher than that for FDG (0.839), indicating that BF-227 is more sensitive as well as more specific than FDG in diagnosing AD.

Discussion

Our group recently developed a novel PET tracer, BF-227, and has reported that this compound is able to selectively detect dense amyloid deposits including senile plaques primarily in the posterior association area of AD patients. In the present study we applied this tracer to MCI cases and concluded that the mean value for the MCI cases with BF-227 was intermittent between AN and AD. Also we clarified that BF-227-PET is a useful technology to distinguish early AD patients from AN compared to FDG–PET.

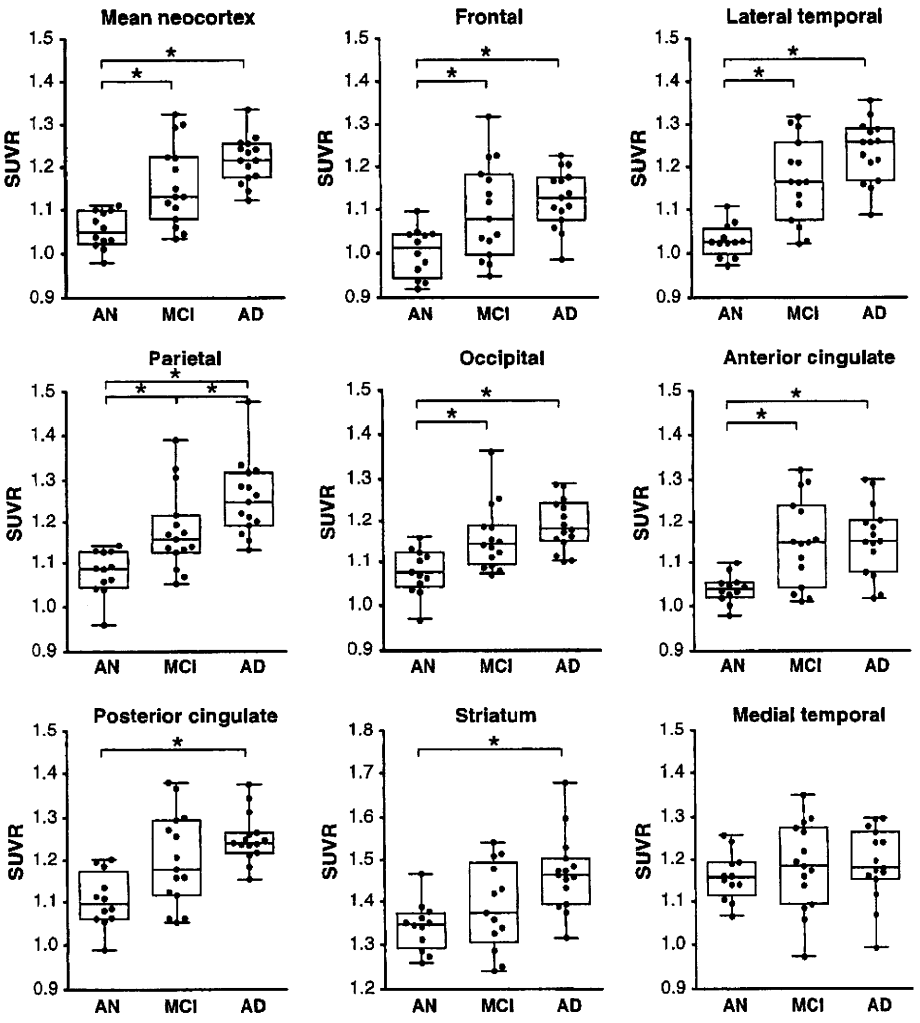

Fig. 2 Box plots of SUVR values with BF-227 PET for AN, MCI and AD. Each *dot* indicates the mean SUVR from "the mean neocortex" and "the eight regions", that is, frontal, temporal, parietal, occipital, anterior cingulate, posterior cingulated, striatum and medial temporal cortex. *Box* indicates interquartile range. *Vertical bars* indicate minimum–maximum range

MCI is now classified into 4 subtypes, that is, amnestic single-domain MCI, amnestic multi-domain MCI, non-amnestic single-domain MCI and non-amnestic multi-domain MCI. The important thing is that MCI (especially amnestic MCI) is regarded as a prodromal state of AD, in other words, a high percentage of MCI subjects are considered to convert to AD. It has been reported that 10–20% of MCI cases are going to convert to AD although only 1–2% of normal elderly convert to AD [21]. The present study concludes that MCI has high levels of [^{11}C]BF-227 retention indicating that senile plaque deposition already advances severely in the stage of MCI before dementia symptoms become obvious. Previous amyloid PET studies using ^{18}F-labeled 2-(1,1-dicyano-propen-2-yl)-6-(2-fluoroethyl)-methylamino-naphthalene (FDDNP) or PIB also indicated significant tracer retention in MCI and AD. Small et al. [24] presented that FDDNP can detect a high signal in MCI by binding not only for amyloid plaques but also tau neurofibrillary tangles, and the retention level for MCI is between AN and AD. On the other hand, several groups reported that about a half of the MCI subjects showed PIB uptake in the AD range, and other MCI subjects indicated retention levels lower than the AD range [12]. A group from Sweden concluded that MCI subjects who converted to AD later showed significantly higher PIB retention compared to non-converting MCI subjects and NC [6]. The present study also revealed higher retention of BF-227 in 60–70% of MCI subjects and in almost all the AD patients. Therefore, the amyloid PET technique is considered to be a highly useful and strong method for early detection of AD patients in the MCI stage. These pieces of information are indispensable in applying new treatment technologies against dementia into the prodromal stage of Alzheimer's disease. In other words, because it is considered that aggregation and deposition of Aβ starts much earlier before patients indicate symptoms of dementia, it is undoubtedly important to detect Aβ deposition as early as

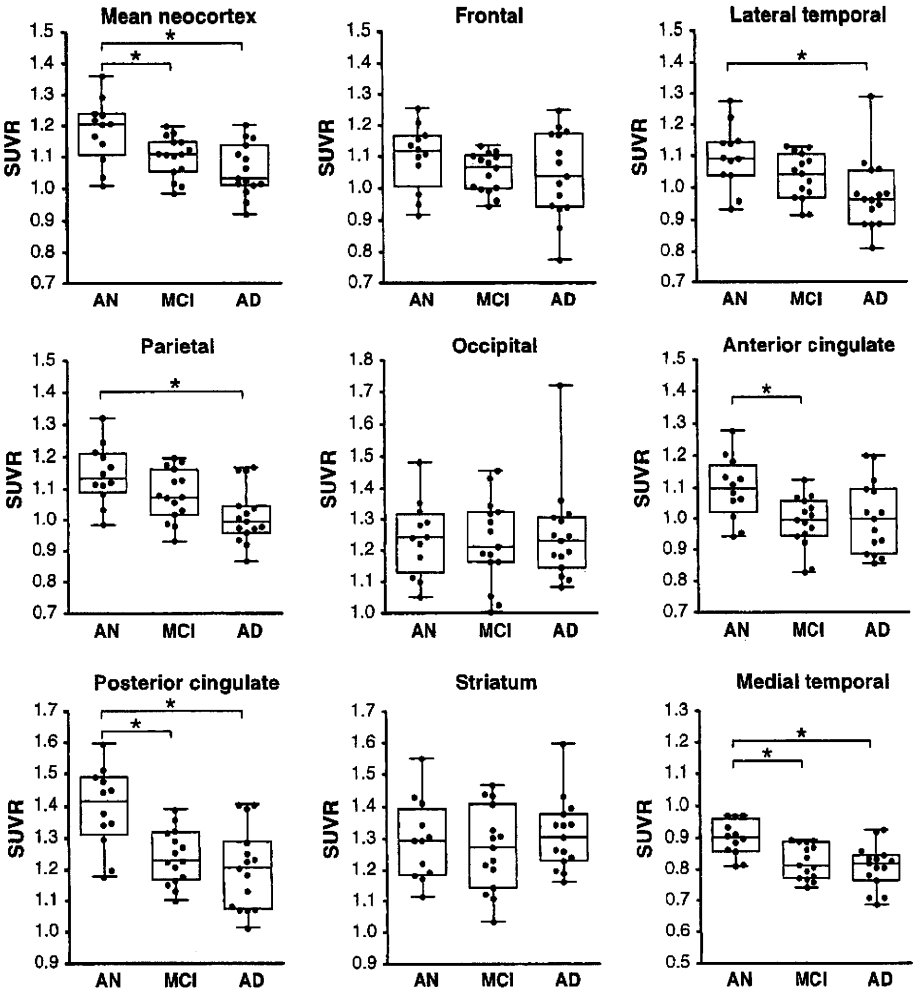

Fig. 3 Box plots of SUVR values with FDG–PET for AN, MCI and AD. Each *dot* indicates the mean SUVR from the mean neocortex and eight cerebral regions, that is, frontal, temporal, parietal, occipital, anterior cingulate, posterior cingulated, striatum and medial temporal cortex. *Boxes* indicate interquartile range. *Vertical bars* indicate minimum–maximum range

Table 2 Comparison of SUVR values of BF-227-PET and FDG–PET

		Mean neo cortex	Frontal	Lateral temporal	Parietal	Occipital	Anterior cingulate	Posterior cingulate	Striatum	Medial temporal
BF-227	AN	1.05 ± 0.04	1.00 ± 0.06	1.03 ± 0.04	1.08 ± 0.05	1.08 ± 0.05	1.04 ± 0.03	1.11 ± 0.07	1.34 ± 0.06	1.16 ± 0.06
	MCI	1.16 ± 0.10*	1.10 ± 0.11*	1.17 ± 0.10*	1.18 ± 0.10*	1.16 ± 0.08*	1.15 ± 0.11*	1.20 ± 0.11	1.41 ± 0.11	1.18 ± 0.10
	AD	1.22 ± 0.06*	1.13 ± 0.07*	1.24 ± 0.07*	1.25 ± 0.09*†	1.19 ± 0.06*	1.16 ± 0.09*	1.25 ± 0.06*	1.47 ± 0.09*	1.19 ± 0.09
FDG	AN	1.18 ± 0.10	1.10 ± 0.11	1.10 ± 0.10	1.15 ± 0.09	1.24 ± 0.12	1.10 ± 0.10	1.39 ± 0.13	1.29 ± 0.13	0.90 ± 0.06
	MCI	1.10 ± 0.06*	1.05 ± 0.06	1.03 ± 0.07	1.08 ± 0.08	1.23 ± 0.14	0.99 ± 0.08*	1.24 ± 0.09*	1.27 ± 0.13	0.82 ± 0.06*
	AD	1.06 ± 0.08*	1.05 ± 0.14	0.98 ± 0.11*	1.01 ± 0.09*	1.25 ± 0.15	1.00 ± 0.12	1.20 ± 0.13*	1.31 ± 0.11	0.81 ± 0.07*

Mean SUVR value for each brain region was obtained from AN, MCI and AD. * $p < 0.05$, versus AN, † $p < 0.05$ versus MCI

possible in order to begin medication to prevent or treat cognitive decline before the manifestations of dementia become clear.

In most PIB positive MCI and AD cases presented by several different laboratories, the frontal cortex showed high PIB retention, although the frontal cortex is not a region where amyloid plaques are predominantly rich in the early stage of AD or MCI according to the autopsy studies [1, 10]. Our newly developed tracer, BF-227, showed relatively high retention in temporal and parietal lobes for MCI and AD compared to the results with PIB. Since it is well known that the functional activity of the parietal lobe decreases in the early stage of AD [16], it is reasonable that the distribution of high BF-227-PET

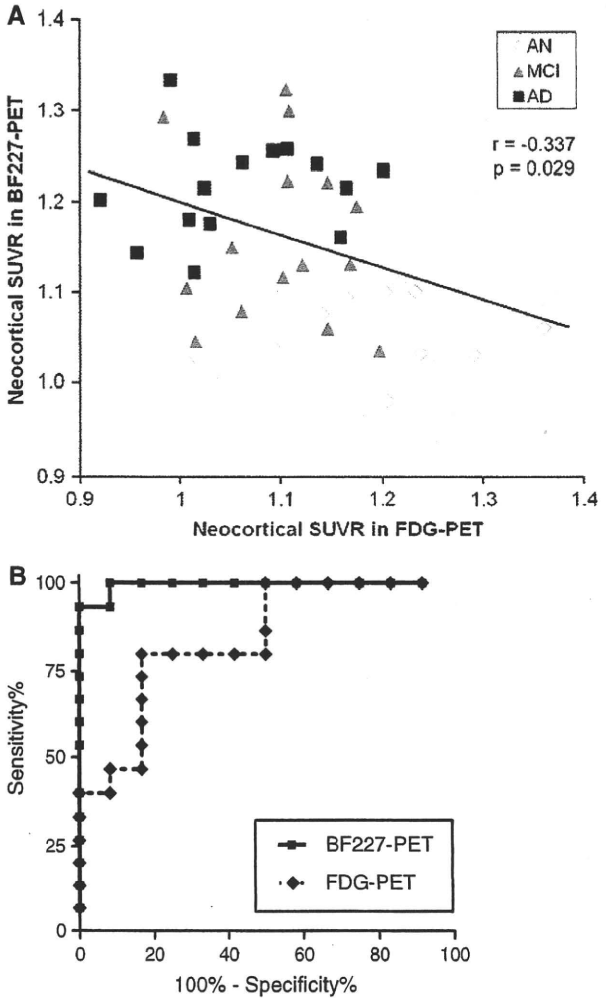

Fig. 4 **a** Relationship between neocortical SUVRs in FDG–PET and BF-227-PET. Neocortical SUVR of FDG–PET for each subject was plotted against neocortical SUVR of BF-227-PET. White, gray and black dots indicate AN, MCI and AD, respectively. **b** Receiver operating characteristic (ROC) curves of BF-227 and FDG–PET. BF-227-PET SUVR in the lateral temporal cortex and FDG–PET SUVR in the posterior cingulate cortex for differentiation between AD and AN

retention is closely related to the area indicating functional deterioration in the early stage of AD or MCI.

Low rCMRglu in AD especially in the posterior cingulate, precuneus, temporoparietal and frontal cortices was reported. FDG–PET has also been used in investigations for MCI, and low rCMRglu in the temporo-parietal and medial frontal cortices and hippocampus was reported as the most prominent predictor of subsequent cognitive decline [2–5]. Our results indicate, however, that amyloid retention detected by BF-227 is more sensitive and specific than FDG–PET for AD diagnosis. Therefore it is reasonable that amyloid PET is more sensitive than FDG–PET for detecting MCI, which is regarded as a prodromal state of dementia or early AD. According to previous autopsy studies with MCI, amyloid plaques were found predominantly in the temporal lobe structure and most amnestic MCI cases showed Braak stage II or III [11, 22]. Furthermore both neurofibrillary tangles and senile plaques were found in nondemented aging and "preclinical" AD, and profound neuronal loss was observed in layer II of the entorhinal cortex [7, 23]. Our results with BF-227 PET for MCI presented here agree with postmortem studies because BF-227 also showed high retention predominantly in the temporal lobe and the retention was intermittent between NC and AD. There are some discrepancies, however, between the results with our BF-227-PET and with autopsy, that is, some cerebral white matter, thalamus and pons showed high retention of BF-227 in MCI, although these regions are usually not rich in senile plaques in the autopsy studies. Although it is considered that the deposition of BF-227 in these regions comes from its non-specific retention by high lipophilicity, it is supposed that more precise studies are needed using more subjects for both PET and autopsy.

We now have to carefully consider the heterogeneity of BF-227 retention in MCI, which was also observed in FDDNP or PIB studies, that is, some subjects show rich retention but others do not. Although it was reported that MCI subjects showing high retention of PIB had a high tendency to convert to AD as we mentioned above [6], the number of subjects they examined was relatively small. Therefore, further careful studies are needed to clarify if the accumulation of amyloid PET probes correlates with the severity of cognitive impairment and a conversion rate to dementia.

Our results using BF-227 for MCI are "continuous" rather than "off/on", "negative/positive" or "dichotomous" signals compared to those with PIB. We speculate that because BF-227 can depict a small difference of amyloid deposition more finely than PIB, the results with BF-227 in MCI are more continuous than those with PIB. Therefore, BF-227 could reveal a degree of senile plaque deposition more precisely and accurately than PIB as far as in cases with MCI.

We would like to conclude that our newly developed amyloid PET tracer, BF-227, can detect amyloid aggregation and deposition in MCI cases and the PET signal intensity for MCI was intermittent between NC and AD. Results obtained with BF-227 PET are significantly more sensitive and specific than FDG–PET in diagnosing AD. As far as the retention pattern in the frontal and parietal cortices, BF-227 more accurately reflects senile plaque deposition observed in the autopsy studies than PIB does. Therefore, BF-227 PET should be an invaluable tool for diagnosis of AD in the early stage. Finally, we recently developed a novel probe, which has similar structure to BF-

227, labeled with F-18, and applied it to living humans. We have finished more than 20 cases so far and obtained similar results to BF-227.

Acknowledgments This study was supported by the Program for the Promotion of Fundamental Studies in Health Science by the National Institute of Biomedical Innovation, the Special Coordination Funds for Promoting Science and Technology, the Industrial Technology Research Grant Program from the New Energy and Industrial Technology Development Organization of Japan, Health and Labour Sciences Research Grants for Translational Research from the Ministry of Health, and the Ministry of Education, Culture, Sports and Technology. We appreciate technical assistance of Dr. Shoichi Watanuki and Dr. Yoichi Ishikawa in the clinical PET studies and Dr. Motohisa Kato in the imaging analysis.

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Invited Review for the 90th Anniversary

Geriatric Medicine, Japanese Alzheimer's Disease Neuroimaging Initiative and Biomarker Development

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Due to a change in disease spectrum in aged countries, the primary role of geriatricians should be directed to an appropriate management and prevention of 1) cognitive decline and dementia, 2) swallowing and aspiration pneumonia and 3) falls and fractures. Management of dementia constitutes a central part in the practice of geriatric medicine in order to support independence of life in elderly people. The current paradigm of cognitive function-based testing for the diagnosis and treatment of Alzheimer's disease (AD) is going to drastically shift to a biomarker-based test approach, a shift that will correspond to the emergence of disease-modifying drugs. In addition, a new molecular imaging technique that visualizes neuronal protein deposits or pathological features has been developed in Japan and the U.S.A. Based on these achievements, the Alzheimer's Disease Neuroimaging Initiative (ADNI) was proposed and initiated in 2005. The ADNI is a long-term observational study being conducted in the U.S.A., Europe, Australia, and Japan using identical protocols. The objectives of ADNI are: 1) to establish methodology which will allow standard values related to long-term changes in imaging data, such as MRI and PET, in patients with AD and mild cognitive impairment and normal elderly persons; 2) to obtain clinical indices, psychological test data, and blood/cerebrospinal fluid biomarkers to demonstrate the validity of image-based surrogate markers; and 3) to establish optimum methods to monitor the therapeutic effects of disease-modifying drugs for AD. Patient enrollment in the Japanese ADNI has begun in July 2008. Imaging of AD pathology not only acts as a reliable biomarker with which to assay curative drug development by novel pharmaceutical companies, but it also helps health promotion toward AD prevention.

Keywords: geriatric medicine; Alzheimer's disease; Amyloid β-peptide; Biomarker; Amyloid imaging; ADNI
Tohoku J. Exp. Med., 2010, **221** (2), 87-95.

Geriatrician's role and proposal of "Geriatric Triangle"

Geriatric medicine is an independent internal medicine division that is specialized for management of medical problems of elderly people. Despite a fact that elderly people appear healthy, a variable latent organ dysfunction may be present due to a limited residual capacity. A condition referred to as geriatric syndrome is a complex and multi-organ disease especially suffered by elderly people. The geriatric syndrome consists of more than 50 medical conditions such as dementia, depression, delirium, pneumonia, urinary incontinence, osteoporosis and fractures as well as malnutrition, sarcopenia, skin ulceration and renal failure. Importantly, these clinical conditions often occur in combination rather than separately. As illustrated in Fig.1, most important functions which support independence of life in later years are: 1) Thinking and judgments; 2) Eating and swallowing; and 3) Standing and walking. Loss of these basic functions alone or in combination will directly lead to devastating health implications and reduced quality of life. Disturbance of cognitive ability manifests as dementia. Impairment of ordered oropharyngeal functions causes a disturbed swallowing or dysphasia followed by development of aspiration pneumonia. Failure of standing and walking results in repeated falls and fractures. — all being hardly present before the age of 65 but highly prevalent over the age of 75. Moreover, these problems not merely occur in separate occasions but they also are inter-related each other. For example, people with advanced dementia are likely to develop eating problems and aspiration (Nakagawa et al. 1997; Wada et al. 2001; Mitchell et al.

Received March 23, 2010; revision accepted for publication April 5, 2010. doi:10.1620/tjem.221.87
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2009). Repeated episodes of pneumonia will develop disturbed nutrition and dehydration which leads to sarcopenia with an increased risk of falls and fractures (Lang et al. 2010). A long-term bedridden state due to hip or vertebral fractures will result in worsening of dementia (Muir et al. 2009). Conversely, demented patients who were treated by anti-psychotic drugs are associated with an increased risk of falls and fractures (Horikawa et al. 2005). A long-term bedridden state due to hip or vertebral fractures are prone to develop esophageal regurgitation and aspiration (Matsui et al. 2002). Drugs that up-regulate brain dopaminergic function are occasionally beneficial to prevent aspiration pneumonia in the elderly (Yamaya et al. 2001). Here, we propose to term such a closely-related condition as "geriatric triangle" as shown in Fig.1. Patients diagnosed as having geriatric triangle are likely to be placed on a long-term care facility due to reduced quality of life (Sasaki 2008). Therefore, the primary role of geriatricians should be directed to an appropriate management and prevention of geriatric triangle. Moreover, every single geriatrician should be capable of managing the geriatric triangle beyond a scope of each organ specialist (Sasaki 2008). Hence, primary targets of geriatric medicine may include assessment and treatment of 1) cognitive decline and dementia, 2) swallowing and aspiration pneumonia and 3) falls and fractures. On the other hand, it is unlikely as a primary role of geriatrician only to manage elderly people with diseases which are spanning entire stages of life. Such diseases, for example, hypertension and diabetes mellitus, can be taken care of by each organ specialist. Due to a change in disease spectrum in aged countries, it should be emphasized that geriatric medicine has become a separate and independent practice division from other organ-specialized fields of internal medicine.

Current scientific approach toward understanding of Alzheimer's disease (AD) pathogenesis

Alzheimer's disease (AD) deprives sufferers of variable life-supporting functions that are necessary for independence in the later years of life. Development of AD leads to parting from society. Care-taking families sacrifice their quality of life and their mental and physical burdens are immeasurable. Loss of personality due to alteration of brain function while physical appearance remains the same is horrible and miserable. As an essential domain of geriatric triangle as described in Fig. 1, prevalence of dementia (the number of people with the disease at any one time) doubles for every 5-year age group beyond the age 65. Briefly, dementia hardly develops prior to age 60. However, according to data from Ministry of Health, Labor and Welfare in Japan, the prevalence of dementia is estimated to be 1.5% for age 65-69, 3.6% for age 70-74, 7.1% for age 75-79, 14.6% for age 80-84, 27.3% beyond age 85 (http://www.mhlw.go.jp/english/index.html). The elderly population aged 65 or older is now approximately 22% of the whole population in Japan. Therefore, it is likely that dementia becomes quite common over the age of 65. According to recently conducted community survey, AD is a leading cause of dementia among elderly Japanese population (Yamada et al. 2001; Wada-Isoe et al. 2009). The rapid increase in the number of AD patients can be a consequence of a rapid increase in human life span. In Japan, an average life span in 1947 was 50.6 years for men and 53.9 years for women. Surprisingly, that was 79.3 years for men and 86.1 years for women in 2008. It is possible that AD is only encountered when the nation reaches a sufficiently aged society. Furthermore, AD is a major factor in increasing national medical expense. It is a universal desire to find a way to control AD. The U.S.A. calls the rapid increase in

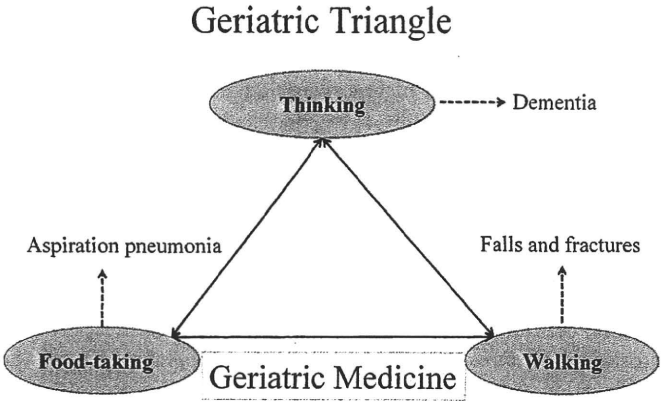

Fig. 1. Proposed concept of geriatric triangle.
At least three physical and mental functions are needed to support independence of life in elderly people. They are 1) Food-taking ability, 2) Standing and walking, and 3) Thinking and judgments. Loss of these basic functions alone or in combination will lead to devastating health implications and reduced quality of life through a vicious circle. Here, we propose to term such a vicious circle as "geriatric triangle". Geriatric triangle constitutes a major part of geriatric syndrome. Therefore, each geriatrician should be capable of managing the geriatric triangle beyond a scope of each organ specialist.

AD patients and concomitant pressure on federal budget a "National Crisis" which illustrates the seriousness of the problem (A National Alzheimer's Strategic Plan, 2009).

Understanding of pathogenesis of AD has markedly progressed in the last 3 decades. Pathological changes of AD occur gradually initially in cognitively normal people with dementia representing the end stage of many years of accumulation of amyloid β-peptide (Aβ). Aβ was first sequenced from meningeal blood vessels of AD brains (Glenner&Wang 1984). A year later, the same peptide was discovered as the primary components of senile plaques (Masters et al. 1985). Shortly after these earlier findings, cloning of the gene encoding amyloid β-peptide precursor protein (APP) and its localization to chromosome 21coupled with the recognition that Down's syndrome (trisomy 21) leads invariably to AD neuropathology set a initial hypothesis that Aβ is a primary driving force in the pathogenesis of AD. The other neuropathological features that are characteristic of AD include neurofibrillary changes and neuron death. Spatial distribution of senile plaques differs from that of neurofibrillary changes (Arriagada et al. 1992a; Arriagada et al. 1992b). A major building block of neurofibrillary changes was shown to be abnormally phosphorylated tau (Lee et al. 1991). According to the amyloid hypothesis, cortical Aβ accumulation causes all of the disease process associated with AD including microglial and astroglial activation, synaptic injury, oxidative injury followed by abnormal tau phosphorylation and eventually loss of neurons and dementia (Hardy and Selkoe 2002). The amyloid hypothesis also tells us that control of amyloid deposition would achieve success to control AD. There have been several conceptually important observations that strongly support the amyloid hypothesis. First, we occasionally see Aβ-positive but tau-negative brains from cognitively normal elderly people in autopsy samples, suggesting that Aβ deposition predates tau deposition (Arai et al. 1990). This time framework was further evidenced by the observation that Aβ-positive senile plaques occur at age 30's, whereas tau-positive neurofibrillary changes are seen only after the age of 40 in the brains afflicted with Down's syndrome (Mann et al. 1989). Thirdly, genetic mutations causing autosomal dominant familial AD were discovered in the APP gene clustering at or very near the sites that are normally cleaved by proteases called β or γ-secretases (Goate et al. 1991). These mutations enhance proteolytic processing of APP to generate amyloidogenic Aβ (Citron et al. 1992). Other AD-causing mutations in PS-1 and PS-2 gene also enhance generation of amyloidogenic Aβ by changing proteolytic processing of APP (Scheuner et al. 1996). Finally, a distinct Aβ species ending at amino acid 42 (Aβ42) is highly amyloidogenic, and there was a uniform pattern of Aβ42 deposition as an initial event of pathology either in non-demented, AD or Down's syndrome patients (Iwatsubo et al. 1994). As illustrated in Fig. 2, we can use a hypothetical assumption to think about the progression of AD. Namely, assuming that memory loss became noticeable at the age 70 followed by progression of multiple cognitive decline and behavioral problems at the age of 75. The patient was eventually diagnosed as suffering AD. In such an instance, we can assume that accumulation of cerebral Aβ may have started at around 50 years of age followed by intracellular accumulation of tau in the form of neurofibrillary changes as well as neuron death may have started at approximately 60-65 years of age. Therefore, it should be emphasized that there is an approximately 20-year time lag between the initiation of amyloid protein deposition and onset of the earliest clinical manifestations of dementia in AD. During this lag-period, individuals are cognitively normal but they are not aware of what changes are taking place in their brains. We assume that such individuals would ultimately develop AD if he or she lived long enough. Furthermore, a prodromal stage of AD often referred to as mild cognitive impairment (Petersen et al. 2009) is characterized by onset of mildest cognitive symptoms despite a massive neuron loss in vulnerable cortical areas (Gómez-Isla et al. 1997). Hence, there is an extremely high need for development of methods that simply and reliably detect amyloid and tau deposits. One such approach is a recently developed molecular imaging technique called "amyloid imaging".

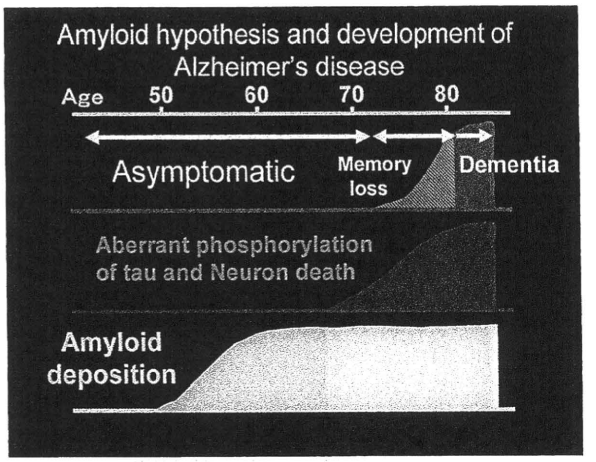

Fig. 2. Hypothetical scheme of progression of AD from amyloid deposition to development of dementia.
It is noteworthy that brain amyloid continues to be accumulating towards the onset of AD during which subjects are not aware of what changes are taking place in their brains. When subjects are first symptomatic, abundant neurofibrillary changes and a massive neuron death have already begun in vulnerable brain regions such as hippocampal or entorhinal cortex. Original description was made by Yasuo Ihara.

A paradigm shift in the diagnosis and treatment of AD

Fig. 3 illustrates a superimposition of the diagnostic and treatment framework in the context of the hypothetical amyloid cascade described above. AD has so far been diag-

nosed clinically only by demonstrating "cognitive decline" which has progressed to a stage that is sufficient enough to disturb independent social or occupational life. It is likely that cognitive decline is associated with a massive neuron death that exceeds so-called "cognitive reserve capacity" (Stern 2009). In addition to cognitive testing, two other diagnostic techniques including magnetic resonance imaging (MRI) and fluorodeoxyglucose (FDG)-PET are currently in common use to demonstrate a mass of dead nerve cells directly or indirectly. Symptomatic drugs such as donepezil hydrochloride and memantine hydrochloride are best considered at this point. However, a dramatic improvement of memory function cannot be expected since disturbance of episodic memory is based upon a massive loss of hippocampal and entorhinal cortical neurons. Accordingly, if we assume that AD represents chronic effects of a long-standing imbalance between Aβ production and Aβ clearance and this imbalance causes all existing events in the downstream of Aβ, a special attention should be directly paid to amyloid and tau depositions in the development of preventive strategies. If we are successful in developing diagnostic methodologies to detect amyloid or tau deposition before a massive neuron death occurs, such approaches will make a great contribution to developing a disease-modifying or curative treatment that directly targets amyloid and also tau. A paradigm of cognitive function-based testing for the diagnosis and treatment of AD is going to drastically shift to a biomarker-based test approach in accordance with the emergence of disease-modifying drugs. Hope for prevention of AD would be potentially carried out. As mentioned later, the Alzheimer's Disease Neuroimaging Initiatives (ADNI) will change paradigm of diagnostic and treatment of AD drastically with biomarkers as a bridging role in the paradigm shift.

Biomarkers with a bridging role in the paradigm shift

In general, biomarkers of AD are defined as indicators of specific features that characterize AD in vivo. Either biochemical or imaging biomarkers are expected to provide potentially diverse purposes as summarized elsewhere (The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association & NIAWG 1998; Frank et al. 2003; Shaw et al. 2009). First, biomarkers will support preonset diagnosis. As demonstrated in Fig. 2 and 3, AD pathology has already started with abundant amyloid pathology even though individuals are otherwise normal and are still independent in their daily living activities,. This stage can be an ideal therapeutic time point in which disease-modifying or curative drugs should be indicated before neurodegenerative cascade is triggered. Such biomarkers will enable us to move from disease modification to prevention of AD. Second purpose is evaluation of disease severity. Currently, severity or clinical stage of AD is evaluated by neuropsychological testing. However, neuropsychological test results are likely to vary due to the patient's physical condition on the day of the test and experience of the examiners. In a study involving 192 AD patients performed by Jack et al., the annual change in ADAS-Cog score in mild to moderate AD was 4.25 ± 7.2 (mean ± S.D.) points, while the yearly change in hippocampal volume on MRI in the same patients was −234 ± 144 (mean ± S.D.) mm^3 (Jack et al. 2003; Petersen et al. 2005). The SD, representing variation of the values, of the hippo-

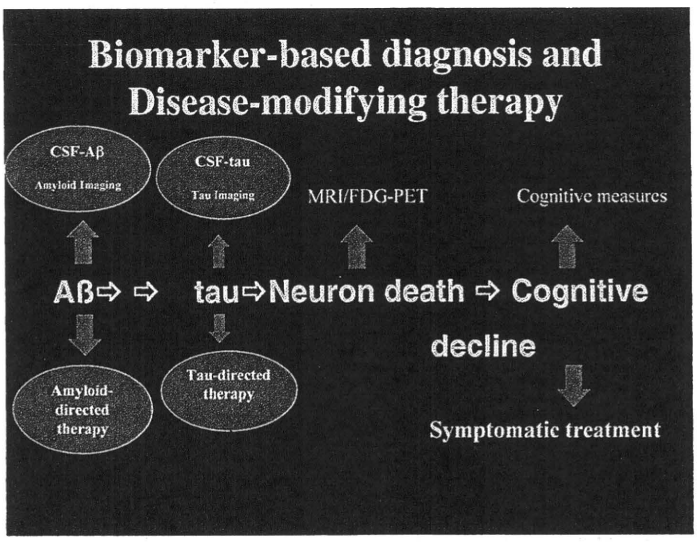

Fig. 3. Strategies for new diagnostic and therapeutic approaches for AD are presented based on amyloid hypothesis.
This figure illustrates a superimposition of the diagnostic and treatment framework in the context of the hypothetical amyloid cascade as described in Fig. 2. In the hypothesis, amyloid is located upstream probably due to a causative agent of AD. Therefore, amyloid imaging is quite attracting because this technology will facilitate both detection and intervention that targets amyloid. If tau imaging would also be possible, tau-targeting therapy might be considered.

campal index was only 0.6 times the mean, while that of ADAS-Cog was 1.7 times. Since image processing is a uniform mechanical task, variation of the imaging biomarker should be small. Sensitive biomarkers which reliably and objectively reflect changes in lesions, even though the effect size is small, are expected to be used analogously to commonly used laboratory test indices for evaluation of the disease severity in clinical practice such as C-reactive protein in inflammatory diseases, serum transaminase levels in liver diseases as well as serum creatinine kinase levels in muscular diseases. Thirdly, we need biomarkers that support evaluation of therapeutic effects. Several classes of amyloid-reducing drugs such as γ-secretase inhibitors (De Strooper et al. 2010) and amyloid immunization therapy (Tabira 2010) might become available in the near future. For the development of these therapeutic drugs, development of methodology to objectively access "decrease or removal of amyloid" is necessary. For example, when the brain amyloid level is reduced by a novel treatment, the biomarker levels are expected to return closer to normal range. Ideal biomarkers may also provide important information regarding the timing of treatment initiation, discontinuation and changing of drug treatment. However, it may be unlikely that a single biomarker meets all conditions described above, and it may be more realistic to prepare a combination or panel of several different biomarkers.

Since therapy is likely to be most effective at or before symptom onset, early or pre-symptomatic detection of AD is highly desirable before neurodegeneration becomes obvious. Thus, there is a great need for blood and CSF biomarkers that substantially aid tracking disease progression of AD and eventually promoting prevention strategy. As reviewed elsewhere (The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association & NIAWG 1998; Frank et al. 2003), ideal AD biomarkers should detect a fundamental feature of AD neuropathology, be validated in autopsy confirmed cases, have a diagnostic sensitivity > 80% for detecting AD and a specificity of > 80% for distinguishing AD from other dementias. Moreover, assays using AD biomarkers should be reliable, reproducible, non-invasive, simple to perform and inexpensive. Further, validation of AD biomarkers requires confirmation by at least 2 independent studies from qualified investigators published in peer-reviewed journals. Tau and Aβ are major components of the two neuropathological hallmarks of AD (tangles and plaques respectively), and they are the most intensively studied candidate AD biomarkers where they are best studied in cerebrospinal fluid (CSF) using extensively characterized ELISAs (Arai et al. 1995; Arai et al. 1997; Arai et al. 1998; Tomita et al. 2007). A recent examination of > 100 subjects with autopsy-confirmed diagnoses reached a conclusion that elevated CSF tau levels are associated with the presence of AD pathology and CSF Aβ42 levels are decreased in AD (Clark et al. 2003). Currently, it is widely accepted that biomarkers of brain amyloid burden are reductions in CSF Aβ42 and increased amyloid PET tracer retention (Fagan et al. 2006; Jack et al. 2010). As shown in Fig. 2, after a lag period, which varies from patient to patient, neuronal dysfunction and neurodegeneration become the dominant pathological processes. Biomarkers of neuronal injury and neurodegeneration are increased CSF tau and structural MRI measures of cerebral atrophy (Arai et al. 1995). Neurodegeneration is accompanied by synaptic dysfunction, which is indicated by decreased FDG-PET (Jack et al. 2010).

Development and clinical applications of amyloid imaging

Amyloid imaging is currently considered to be the most promising candidate biomarker since it meets many possible conditions of an ideal biomarker as described above. The most difficult hurdle for clinical application of this technology is to find a probe with following excellent characteristics: 1) it should selectively bind to Aβ aggregates with β-sheet-structure; 2) it should readily penetrate the blood-brain barrier (BBB) while being rapidly cleared off from the brain in the absence of the target; 3) the labeled form should not lose the characteristics of the mother compound. In our experience, enhancing one of several necessary characteristics causes loss in another, requiring extensive adjustment.

Although brain Aβ deposits are still well beyond the resolution of conventional neuroimaging techniques such as MRI, the density of these deposits in the brain tissue can be visualized through specific radiotracer and positron emission tomography (PET). The first compound to emerge as an amyloid-imaging agent was Chrysamine-G (Klunk et al. 1995). This compound shows similar binding characteristics to Congo-red, but unfortunately, due to its limited BBB permeability, there was no use as a clinical PET tracer. A marked progression in the development of amyloid-imaging tracers was made by the development of 2-(1-{6-[(2-[^{18}F]fluoroethyl)(methyl) amino]-2-naphthyl}ethylidene) malononitrile ([^{18}F]FDDNP) (Agdeppa et al. 2001). This compound is highly lipophilic and can easily cross BBB, and has been used in human PET studies (Shoghi-Jadid et al. 2002; Small et al. 2006; Barrio et al. 2008). However, this agent has some limitations in its practical use due to its low signal-to-background ratio (Tolboom et al. 2009). Currently, the most successful amyloid-binding agent is a thioflavin-T derivative, N-methyl-[^{11}C] 2-(4′-methylaminophenyl)-6-hydroxybenzothiazol ([^{11}C]PIB) which has been shown to possess a high affinity for Aβ fibrils (Klunk et al. 2003; Mathis et al. 2003; Klunk et al. 2004). An autoradiographic study using AD brain sections revealed that [^{11}C]PIB, in addition to binding to the classical fibrillar Aβ plaques, also binds to a range of Aβ containing lesions including diffuse plaques and cerebrovascular amyloid angiopathy (Lockhart et al. 2007). In vitro binding studies indicated that PIB preferentially binds to Aβ1-42 fibrils with high affinity (Klunk et al. 2003) with a negligible binding to α-synuclein and tau (Lockhart et al. 2007; Fodero-

Tavoletti et al. 2007). The [^{11}C]PIB retention in the neocortical areas is correlated with the Aβ plaque load (Bacskai et al. 2007; Ikonomovic et al. 2008) with an inverse relation to CSF Aβ42 levels (Fagan et al. 2006). The frequency of cognitively normal individuals with positive PIB binding rose in an age-dependent manner from 0% at ages 45-49 years to 30.3% at ages 80-89 years. (Rowe et al. 2007; Morris et al. 2010). Further, CSF tau and phospho-tau$_{181}$ increased with cortical PIB binding in cognitively normal individuals (Fagan et al. 2009). However, there is currently no evidence of how frequently PIB-positive normal individuals will convert to develop dementia or how long is the interval between the detection of significant Aβ burdens and the onset of dementia. Longitudinal amyloid imaging studies are needed to demonstrate the reality of amyloid hypothesis via looking at relation between amyloid deposition and temporal AD progression.

Benzoxazole derivatives are also promising alternatives as amyloid-imaging probes (Okamura et al. 2004). A PET study using the ^{11}C-labeled benzoxazole derivative 2-(2-[2-dimethylaminothiazol-5-yl]ethenyl)-6-(2-[fluoro]ethoxy) benzoxazole (BF-227) demonstrated significantly higher retention of this tracer in cerebral cortices of AD patients compared to the majority of healthy elderly subjects (Kudo et al. 2007). The retention of this tracer in cerebral cortices of mild cognitive impairment patients was intermediate between AD and healthy normal subjects (Waragai et al. 2009; Furukawa et al. 2010). A voxel-by-voxel analysis demonstrated a higher retention of [^{11}C]BF-227 in the posterior association cortex of AD patients. The pattern of this distribution corresponds well with the distribution of neuritic plaques in postmortem AD brains (Okamura et al. 2009). These findings suggest [^{11}C]BF-227 may be distinct from [^{11}C]PIB in detecting different populations of amyloid deposits. In addition, glucose metabolism demonstrated by FDG-PET was negatively correlated with that of BF-227, suggesting that extracellular amyloid surrounds synapses and impairs neuronal function (Furukawa et al. 2010). In my personal view, a highly expected value of amyloid imaging may be its capability to monitor treatment effects in PIB or BF-227 positive normal individuals who have received amyloid-reducing therapies (Rinne et al. 2010). The [^{11}C]-labeled form has a short half-life (20.4 minutes) and its synthesis requires a facility capable of radioisotope synthesis using a cyclotron, whereas the [^{18}F] -labeled form has a longer half-life (109.7 minutes), which may be amenable for delivery to various sites. Therefore, the [^{18}F]-labeled compounds, for example, [^{18}F]AV-45 will probably be a standardized agent for future clinical uses (Personal communication from Skovronsky D).

Future prospects of the Japanese ADNI

Development of curative molecular targeting therapy for AD has rapidly progressed centering mainly in work done by U.S. pharmaceutical companies. Clinical trials of symptomatic treatments currently on the market could be completed within about 6 months, but planned disease-modifying drugs to delay progression of AD may require trial durations of at least one year or longer to confirm sufficient drug effect. Development of a surrogate biomarker which reflects the pathology of the disease and monitors its progression may be desperately needed for conducting long-term clinical trials. Based on this consideration, an observational clinical study called "The Alzheimer's Disease Neuroimaging Initiative (ADNI)", was proposed and initiated in the U.S.A. in 2005 (Mueller et al. 2005; http://www.adni-info.org/ ; http://www.loni.ucla.edu/ADNI/). ADNI is a non-randomized long-term observational study undertaken in the U.S.A., Europe, Australia, and Japan using an identical protocol in each participant nation. Japanese ADNI (J-ADNI) is planning to follow 300 patients with MCI for 3 years, 150 patients with early AD for 2 years, and the other 150 normal subjects for 3 years in a cooperative study of a total of 38 facilities nationwide with sufficient experience in the management of dementia (http://www.j-adni.org/). The principle investigator is Professor Takeshi Iwatsubo at University of Tokyo. The study objectives are: 1) to establish methodology that will determine standard values related to long-term changes in image data, such as MRI and PET, in AD and MCI patients and normal elderly persons; 2) to simultaneously collect clinical indices, psychological tests, and blood/cerebrospinal fluid biomarkers to demonstrate the validity of image surrogate markers, and 3) to establish the optimum method to monitor therapeutic effects of curative drugs (disease-modifying drugs) for AD, for which analyses of the following observation items are prioritized: 1) Rate of conversion from MCI to AD, 2) rates of whole brain and hippocampus volume changes via MRI, 3) rates of change in blood and cerebrospinal fluid biomarkers, and 4) rate of change in glucose metabolism on FDG-PET. In addition, baseline amyloid PET scans are given to subjects who agreed it in J-ADNI. We hope that J-ADNI project promotes long-delayed improvements of Japanese infrastructure of medical care system for dementia. It is inadvisable for Japanese medical society to ignore that in the U.S.A. a paradigm shift in AD from 'cognitive measures-based to biomarker-based' has begun after deliberation and discussion on subjects such as clinical trial efficiency and cost reduction. Many different curative drugs are under development by pharmaceutical manufacturers, and global clinical trials of these new drugs are ongoing.

In J-ADNI, firstly, several of Japanese version of the cognitive test batteries were revised by Sugishita M. et al. to normalize the relative difficulty and to enhance maximum compatibility of the test with World Wide ADNI and later for global clinical trials of new drugs. The first patient was successfully enrolled at the National Center of Neurology and Psychiatry in July 2008. More than 330 patients have already been enrolled as of March 10, 2010. The consent rate to FDG-PET, amyloid PET, and sampling of cerebrospinal fluid was obtained from 80, 44, and 40% of the participants, respectively. We will attempt to increase the

number of patients enrolled and the rate of consent to biomarker sampling, aiming at a great success of J-ADNI and World Wide ADNI together.

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doi:10.1093/brain/awq091 Brain 2010: 133; 1772–1778 | 1772

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In vivo visualization of α-synuclein deposition by carbon-11-labelled 2-[2-(2-dimethylaminothiazol-5-yl)ethenyl]-6-[2-(fluoro)ethoxy]benzoxazole positron emission tomography in multiple system atrophy

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The histopathological hallmark of multiple system atrophy is the appearance of intracellular inclusion bodies, named glial cytoplasmic inclusions, which are mainly composed of α-synuclein fibrils. *In vivo* visualization of α-synuclein deposition should be used for the diagnosis and assessment of therapy and severity of pathological progression in multiple system atrophy. Because 2-[2-(2-dimethylaminothiazol-5-yl)ethenyl]-6-[2-(fluoro)ethoxy] benzoxazole could stain α-synuclein-containing glial cytoplasmic inclusions in post-mortem brains, we compared the carbon-11-labelled 2-[2-(2-dimethylaminothiazol-5-yl)ethenyl]-6-[2-(fluoro)ethoxy] benzoxazole positron emission tomography findings of eight multiple system atrophy cases to those of age-matched normal controls. The positron emission tomography data demonstrated high distribution volumes in the subcortical white matter (uncorrected $P<0.001$), putamen and posterior cingulate cortex (uncorrected $P<0.005$), globus pallidus, primary motor cortex and anterior cingulate cortex (uncorrected $P<0.01$), and substantia nigra (uncorrected $P<0.05$) in multiple system atrophy cases compared to the normal controls. They were coincident with glial cytoplasmic inclusion-rich brain areas in

Received January 13, 2010. Revised March 15, 2010. Accepted March 17, 2010. Advance Access publication April 29, 2010

multiple system atrophy and thus, carbon-11-labelled 2-[2-(2-dimethylaminothiazol-5-yl)ethenyl]-6-[2-(fluoro)ethoxy] benzoxazole positron emission tomography is a promising surrogate marker for monitoring intracellular α-synuclein deposition in living brains.

Keywords: glial cytoplasmic inclusion; Lewy body; β-amyloid; Parkinson's disease; Pittsburgh compound B

Abbreviations: BF-227 = 2-[2-(2-dimethylaminothiazol-5-yl)ethenyl]-6-[2-(fluoro)ethoxy]benzoxazole; MSA = multiple system atrophy; PIB = Pittsburgh compound B

Introduction

Multiple system atrophy (MSA) is a sporadic, progressive neurodegenerative disease characterized by variable severity of parkinsonism, cerebellar ataxia, autonomic failure and pyramidal signs. Although MSA was originally described as three separate diseases [olivopontocerebellar atrophy (Dejerine and Thomas, 1900), striatonigral degeneration (van der Eecken *et al.*, 1960) and Shy–Drager syndrome (Shy and Drager, 1960)], they are currently classified into a single disease that consists of MSA with predominant parkinsonism and MSA with predominant cerebellar ataxia (Gilman *et al.*, 1999). The histopathological hallmark of MSA, glial cytoplasmic inclusions, comprises mainly insoluble fibrils of phosphorylated α-synuclein (Wakabayashi *et al.*, 1998). Thus, it is suggested that the MSA is in the family of α-synucleinopathies (Marti *et al.*, 2003) including Parkinson's disease and dementia with Lewy bodies, which are characterized by the presence of Lewy bodies, representing other brain inclusions composed of α-synuclein.

Previous neuropathological studies indicated that the appearance of glial cytoplasmic inclusions preceded the clinical onset of MSA (Fujishiro *et al.*, 2008) and the amount of α-synuclein deposition correlated with the disease progression (Wakabayashi and Takahashi, 2006). Therefore, it is plausible that the formation of α-synuclein deposits plays a key role in neurodegeneration, and that compounds that inhibit this process may be therapeutically useful for MSA and other α-synucleinopathies. In fact some compounds, including antioxidants (Ono and Yamada, 2006) and non-steroidal anti-inflammatory drugs (Hirohata *et al.*, 2008), were reported to have potent anti-fibrillogenic and fibril-destabilizing effects on aggregated α-synucleins, and received much attention as possible new therapeutic agents (Ono and Yamada, 2006; Hirohata *et al.*, 2008). Detection of α-synuclein deposition *in vivo* could theoretically allow early diagnosis even at the presymptomatic stage, as well as assess disease progression and possible therapeutic effects in the living brain of patients with MSA.

Although Pittsburgh compound B (PIB) and other compounds were reported to be useful in detecting senile plaques *in vivo*, to our knowledge, there were no imaging probes currently available for *in vivo* detection of α-synuclein deposition. Recently, 2-[2-(2-dimethylaminothiazol-5-yl)ethenyl]-6-[2-(fluoro)ethoxy] benzoxazole (BF-227), known as a positron emission tomography (PET) probe for *in vivo* detection of dense β-amyloid deposits in humans (Kudo *et al.*, 2007), was reported to bind with synthetic α-synuclein aggregates as well as β-amyloid fibrils *in vitro* (Fodero-Tavoletti *et al.*, 2009). In the present study, we demonstrated that BF-227 could stain α-synuclein-containing glial cytoplasmic inclusions in post-mortem tissues and moreover, that a PET study with carbon-11-labelled BF-227 ([11C]-BF-227) could detect α-synuclein deposits in the living brains of patients with MSA.

Materials and methods

Neuropathological staining

Brain specimens

The subjects of the first part of the study were nine autopsy cases, including three with Parkinson's disease, three with dementia with Lewy bodies and three with MSA. The above diagnoses were confirmed both clinically and histopathologically. Brain tissues taken from the temporal cortex and substantia nigra of patients with Parkinson's disease and dementia with Lewy bodies, and pontine base of patients with MSA, were fixed in 20% buffered formalin for 72 h at 4°C, and vibratome sections (50 μm thick) were prepared.

Fluorescence and immunohistochemical analysis

BF-227 was dissolved in 50% ethanol containing 5% polysorbate (Tween 80; Wako, Osaka, Japan). The sections were slide mounted, incubated in 100 μM BF-227 for 30 min, dipped three times in phosphate buffer, and coverslipped with non-fluorescent mounting medium (Vectashield, Vector Laboratories, Burlingame, CA, USA). Fluorescence images were visualized using an Olympus Provis fluorescence microscope (Olympus, Tokyo, Japan) at wavelength 400 nm. After photographing fluorescent structures, BF-227-labelled sections were immunostained with primary antibodies against phosphorylated α-synuclein (#64; Wako). For phosphorylated α-synuclein immunohistochemistry, the sections were pre-treated with 99% formic acid for 5 min, then incubated overnight at 4°C with each primary antibody followed by incubation with the biotinylated secondary antibodies and the avidin–biotin–peroxidase complex (Vectastain ABC kit, Vector Laboratories). Diaminobenzidine was used as the chromogen.

PET study

Subjects

Eight patients with probable MSA and eight age-matched normal subjects were studied to examine the distribution of [11C]-BF-227 in the brain. All probable MSA patients were diagnosed on the second consensus criteria for probable MSA (Gilman *et al.*, 2008). Table 1 summarizes the clinical features of these patients. There were no significant differences in age, disease duration and unified MSA rating scale score between the MSA with predominant parkinsonism

Table 1 Subject profile

	Normal controls	MSA		
		Total	MSA-P	MSA-C
n	8	8	4	4
Gender (F/M)	4/4	4/4	1/3	3/1
Age (years)	64.3 ± 5.90	57.4 ± 10.1	60.5 ± 11.1	54.3 ± 9.50
Duration (years)		1.50 ± 0.54	1.75 ± 0.50	1.25 ± 0.50
UMSARS score		36.1 ± 8.87	41.5 ± 9.39	30.8 ± 4.27

Data are mean ± SD.
MSA-P = MSA with predominant parkinsonism; MSA-C = MSA with predominant cerebellar ataxia; UMSARS = unified MSA rating scale.

subgroup and the MSA with predominant cerebellar ataxia subgroup. The normal control group comprised volunteers without impairment of cognitive and motor functions who had no cerebrovascular lesions on magnetic resonance imaging. The study protocol was approved by the Ethical Committee of Tohoku University Graduate School of Medicine, and a written informed consent was obtained from each subject after being given a complete description of the study.

Radiosynthesis of [^{11}C]-BF-227

BF-227 and its N-desmethylated derivative (a precursor of [^{11}C]-BF-227) were custom-synthesized by Tanabe R&D Service Co. (Tokyo) (Kudo *et al.*, 2007). [^{11}C]-BF-227 was synthesized from the precursor by N-methylation in dimethyl sulphoxide using [^{11}C]-methyl triflate (Jewett, 1992; Iwata *et al.*, 2001). After quenching the reaction with 5% acetic acid in ethanol, [^{11}C]-BF-227 was separated from the crude mixture by semi-preparative reversed-phase high-performance liquid chromatography and then isolated from the collected fraction by solid-phase extraction. The purified [^{11}C]-BF-227 was solubilized in isotonic saline containing 1% polysobate-80 and 5% ascorbic acid. The saline solution was filter sterilized with a 0.22 mm Millipore® filter for clinical use. The radiochemical yields were >50% based on [^{11}C]-methyl triflate, and the specific radioactivities were 119–138 GBq/mmol at the end of synthesis. The radiochemical purities were >95%.

PET procedure

The [^{11}C]-BF-227 PET study was performed using a SET-2400W PET scanner (Shimadzu Inc., Japan) under resting condition with eyes closed in a dark room. Following a 68Ge/Ga transmission scan of 300–400 s duration, an emission scan was started soon after intravenous injection of 3.7–8.3 mCi of [^{11}C]-BF-227. A dynamic series of PET scans were acquired over 60 min with 23 frames. Emission data were corrected for attenuation, dead time and radioactive decay. Standardized uptake value images were obtained by normalizing tissue concentration by the injected dose and body mass. Arterial blood samples (1.5 ml) from the radial or brachial artery were collected from each subject at 10 s intervals for the first 2 min, and subsequently at intervals increasing progressively from 1 to 10 min until 60 min after the injection of [^{11}C]-BF-227 except for one subject, from whom arterialized venous blood samples (1.5 ml) from a hand vein heated in a far-infrared mat were collected at the same time intervals. The plasma obtained by centrifugation at 3000*g* for 3 min was weighed and the radioactivity was measured with a well-type scintillation counter. Additional arterial blood samples were obtained at four time points during the study (5, 15, 30 and 60 min) for the determination of radiolabelled metabolites in plasma using high-performance liquid chromatography. These data yielded values of the unchanged fraction of parent radiotracer throughout the time frame of the study. A multi-exponential equation was used to describe this curve and to estimate the parent fraction at each measured plasma curve time point.

PET image analysis

To measure α-synuclein deposition densities in the brain, the distribution volume, the ratio of [^{11}C]-BF-227 concentration in tissue to that in plasma at equilibrium, was calculated by Logan's graphical analysis (Logan, 2000), since BF-227 reversibly binds to α-synuclein depositions (Tashiro *et al.*, 2009). Region of interest analysis was performed to evaluate the regional distribution of [^{11}C]-BF-227. Circular regions of interest were placed on individual axial PET images in the frontal cortex, primary motor cortex, parietal cortex, medial temporal cortex, lateral temporal cortex, occipital cortex, anterior cingulate cortex, posterior cingulate cortex, subcortical white matter, caudate nucleus, putamen, globus pallidus, thalamus, substantia nigra, midbrain tegmentum, pons and cerebellar cortex, referring to the individual magnetic resonance images.

Statistical analysis

Data were expressed as mean ± SD. Differences in distribution volume between normal control and MSA groups were evaluated by one-way analysis of variance followed by Bonferroni's multiple comparison test (GraphPad Prism Software).

Results

Neuropathological staining

In the post-mortem brains with Parkinson's disease, double-labelling immunostaining with BF-227 fluorostaining and anti-phosphorylated α-synuclein antibody demonstrated co-localization of the proteins in Lewy bodies in the substantia nigra (Fig. 1A and B). Strong BF-227 staining was observed in the central core (Fig. 1A). BF-227 was also detected in the cortical Lewy bodies in dementia with Lewy bodies (Fig. 1C and D). In MSA, double-labelling experiments using BF-227 and anti-phosphorylated α-synuclein antibody demonstrated BF-227 fluorescent signal in the most of glial cytoplasmic inclusions in the pontine base (Fig. 1E and F).

PET study

Tissue time activity curves of [^{11}C]-BF-227 in the brain indicated more gradual clearance from the brain in patients with MSA compared with normal subjects following initial rapid uptake of radioactivity (Fig. 2A). Relatively high concentrations of [^{11}C]-BF-227 radioactivity were observed in the subcortical white matter and lenticular nucleus in MSA, in which relatively intense α-synuclein deposits were found in the post-mortem brain (Fig. 2B). [^{11}C]-BF-227 exhibited linear regression curves on Logan plot analysis in all brain regions examined. Since the slopes of the regression lines represent the distribution volume of the tracer, these findings indicated a higher distribution volume of [^{11}C]-BF-227 in MSA than in normal controls (Fig. 2C). The regional distribution volume values were high in the subcortical white matter (uncorrected $P<0.001$), putamen and posterior cingulate cortex