episodic memory (r=0.73, p=0.016) and non-memory scores (r=0.64, p=0.046) and inversely associated with CDR SOB (r=−0.68, p=0.03). There were no associations between hippocampal PIB retention and any cognitive parameter in any of the clinical groups (Fig. 3). There were no significant associations between global PIB retention and cortical grey matter volume in either HC or AD patients. In contrast to what was observed for hippocampal THK523 retention, there was no association between hippocampal PIB retention and hippocampal volume in any of the groups examined (Fig. 3).

Discussion

To the best of our knowledge, this is the first time a selective tau imaging agent has been thoroughly evaluated in human volunteers, assessing its associations with cognition and brain volumetrics, as well as a direct comparison with Aβ imaging using PIB.

Global cortical THK523 binding provided a very robust separation of AD patients from healthy elderly subjects (Cohen's d=3.4). Furthermore, cortical THK523 retention in AD patients followed the reported histopathological brain distribution of PHF-tau in AD [10, 11]. Examination of the brain kinetics of THK523 showed that it presents reversible binding kinetics, reaching apparent steady state about 50 min after injection of the radiotracer. Visual inspection of the THK523 images was hampered by the very high retention in white matter. In addition to the high non-specific binding, previous reports have demonstrated substantial concentrations of PHF-tau in white matter in AD [34, 35], suggesting THK523 retention in white matter might not solely reflect marked non-specific binding, but also some small degree of specific binding. Several factors were taken into account for the selection of the reference region. To date, no report has described tau deposition in the cerebellar cortex in sporadic AD [36]. There were no group differences in cerebellar cortex THK523 SUV, and there was no association between cerebellar cortex THK523 SUV with age in the whole cohort, or with dementia severity in the AD group, further supporting the use of the cerebellar cortex as reference region.

The regional brain distribution of THK523 showed a marked contrast when compared to that of PIB. While the highest PIB retention was observed in frontal, posterior cingulate, caudate and temporal cortices, the highest THK523 retention was observed in the inferior temporal, orbitofrontal, hippocampus, insula and parietal cortices. This was further confirmed by a lack of correlation between PIB SUVR and THK523 SUVR (r=0.04, p=0.90).

SD patients were included in the evaluation of THK523 as pathological controls [31]. Rather than tau aggregates, the vast majority of SD cases have been associated with the aggregation of TDP-43 [2, 31]. SD patients showed neither THK523 nor PIB retention in the brain (Supplementary Tables 1 and 2), suggesting the absence of both Aβ [37] and tau deposits in SD.

Three HC showed high cortical PIB retention (PIB+HC), consistent with previous PIB studies that have reported positive scans in 25–35 % of normal elderly individuals [38]. Despite the limited subsample size the finding is interesting because while cortical THK523 retention in PIB+HC was not significantly different from the cortical retention in PIB− HC, THK523 retention in the hippocampus and insula was significantly higher than in PIB− HC, but not significantly different from AD, suggesting that tau deposition in these regions might precede the dementia of AD [6, 39]. These findings might indicate that the combination of widespread cortical Aβ plus hippocampal tau deposition might not be enough to lead to significant cognitive impairment, requiring tau deposition in polymodal and unimodal association areas of the brain for objective cognitive impairment to be manifest [7, 10, 11].

As with Aβ imaging [40], longitudinal studies will assist in establishing the spatiotemporal patterns of tau deposition and help determine whether or not apparently healthy individuals with substantial hippocampal tau deposition will develop the AD phenotype, thus allowing very early, even preclinical diagnosis of AD, or if hippocampal tau deposits are just an age-associated process and only cortical tau deposition leads to cognitive impairment [39, 41].

In AD, hippocampal THK523 retention was associated with cognitive parameters. Similarly, hippocampal THK523 retention was associated with hippocampal volume in both HC and AD patients. Human post-mortem studies have shown that the density of NFTs strongly correlates with neurodegeneration and cognitive deficits, while Aβ plaque density does not [42, 43], a finding that was further confirmed through Aβ imaging studies [18, 32]. Furthermore, in stark contrast with Aβ plaques, NFTs are usually not present in associating cortical regions in cognitively unimpaired individuals [11, 18, 39].

As was previously reported in vitro [21, 44], several lines of evidence support the notion that THK523 selectively binds to PHF-tau and not to Aβ in vivo: (a) cortical THK523 retention is significantly higher in AD, following the known distribution of PHF-tau in the AD brain; (b) PIB and THK523 show different brain regional distribution patterns; (c) there is no correlation between PIB and THK523 retention; and (d) while hippocampal THK523 retention significantly correlates with cognitive parameters and hippocampal atrophy, hippocampal PIB retention does not.

While this was a first-in-human study, the limited sample size requires cautious interpretation of the findings. Furthermore, while our results suggest that ^{18}F-THK523 can reliably quantify PHF-tau deposition in vivo, there are serious limitations associated with the tracer itself. The high white matter THK523 retention, even if it might reflect some small degree of specific binding, precludes simple visual inspection of the

images and requires additional careful PVC even for a simple semi-quantitative analysis, preventing the use of THK523 in research or clinical settings.

Conclusion

This study has shown that despite selective, non-invasive in vivo assessment of PHF-tau in humans being possible, a single aspect of the in vivo behaviour of a tracer can derail its further development. This highlights the need for careful in vivo proof of concept studies at the initial stages of the development before embarking on more complex quantification approaches involving invasive procedures such as arterial cannulation or engaging in costly phase II studies. Better tau tracers, some of them already being evaluated in humans [23, 25–27], will be required for applications such as monitoring disease progression and assessing efficacy of anti-tau therapy. The development of ^{18}F-THK523 has shown to be a significant step towards the integration of tau imaging with Aβ imaging, moving us towards meeting the desired goal of earlier diagnosis of AD to assist the development of preventative treatments as well as identifying subjects for early therapeutic interventions.

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Conflicts of interest None.

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Authors' contributions

Dr. Villemagne had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis

Drs. Villemagne, Furumoto, Fodero-Tavoletti, Kudo, Rowe and Okamura participated in the design, acquisition, analysis and interpretation of the data and writing of this manuscript.

Study concept and design: Villemagne, Okamura, Kudo, Rowe

Acquisition of data: Villemagne, Rowe, Fodero-Tavoletti, Pejoska, Yates, Piguet, Mulligan

Analysis and interpretation of data: Villemagne, Doré, Rowe, Okamura

Drafting of the manuscript: Villemagne, Okamura

Critical revision of the manuscript for important intellectual content: Villemagne, Furumoto, Rowe, Harada, Fodero-Tavoletti, Piguet, Hodges, Yanai, Masters, Kudo, Okamura

Statistical analysis: Villemagne, Okamura

Study supervision: Villemagne, Okamura

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ORIGINAL ARTICLE: BIOLOGY

Brain accumulation of amyloid β protein visualized by positron emission tomography and BF-227 in Alzheimer's disease patients with or without diabetes mellitus

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Aim: Although diabetes mellitus (DM) is considered to be one of the most consistent risks for developing dementia, it is not known if the pathology in dementia patients with DM is similar to or distinct from typical pathological features of Alzheimer's disease (AD). To discover the mechanism of developing dementia in AD patients with DM in a living state, we studied the distribution of amyloid β (Aβ) protein of diabetic AD patients.

Methods: To evaluate the accumulation of Aβ, we examined 14 normal controls, four diabetic patients with AD and 11 non-diabetic patients with AD by positron emission tomography (PET) using BF-227, a currently developed Aβ tracer.

Results: The analysis of PET images among the three groups showed an abundant aggregated Aβ accumulation in the cerebral cortex of both AD patients with and without DM. The extent and distributions of BF-227 accumulation in diabetic AD patients were not significantly different from these of non-diabetic AD patients.

Conclusion: These results suggest that the degree and extent of Aβ deposition is not significantly different between AD with DM and AD alone. **Geriatr Gerontol Int 2013; 13: 215–221.**

Keywords: Alzheimer's disease, amyloid β-peptides, diabetes mellitus, positron emission tomography.

Introduction

Long-standing lifestyle-related disorders from midlife, such as diabetes mellitus (DM) and hypertension, as well as obesity, are likely to be prominent risk factors for developing dementia and Alzheimer's disease (AD).[1] In fact, it is often found that diabetic patients develop AD in their later stage of life. Several separate community-based studies suggest that DM might increase the risk of dementia and AD,[2] though the underlying mechanisms are still not clearly explained.

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AD is well characterized by an accumulation of misfolded proteins in the aging brain, which results in oxidative and inflammatory damage that in turn leads to energy failure and synaptic dysfunction.[3] In contrast, the impact of DM on the central nervous system (CNS) is not clearly understood.

Three major components related to type 2 DM that might underlie the effect of diabetes on the CNS in the development of AD are insulin resistance, hyperinsulinemia and hyperglycemia.[4] In addition to these three components, several other components are associated with the incidence of dementia or progression of cognitive decline. Whitmer *et al.* reported that severe hypoglycemic events were associated with a greater risk of dementia.[5] In addition, daily acute glucose fluctuations are also reported to be associated with cognitive decline.[6] Leptin, adiponectin and glucagon like peptide-1 (GLP-1) have recently been mentioned as potential factors that

are associated with the development of AD.[7–10] These components are not fully independent of each other, and it is unlikely that the impact of DM on the CNS depends exclusively on a single component. Which components play the major role might depend on the patient's clinical history and the present state of DM.

Each of these components are thought to act on several different pathways that are important in the pathophysiology of AD, either indirectly, through inflammation or the development of vascular disease, or directly, through effects on amyloid and tau metabolism, and the formation of advanced glycation end-products (AGE).[11] (Fig. 1)

Autopsy results in an epidemiological study concluded that macroscopic brain infarcts are more common in people with DM than those without the disorder, as well as microvascular changes.[12] In contrast, the reported incidence of Alzheimer's pathology in the brains of people with diabetes varies between studies. There are several contradictory papers reporting the relationship between DM and AD. Beeri *et al.* have reported that type 2 DM is inversely associated with AD pathology; that is, diabetic patients with dementia have a significantly lower density of senile plaques than non-diabetic patients with dementia.[13] Matsuzaki *et al.* reported that hyperinsulinemia and hyperglycemia caused by insulin resistance are positively associated with the pathology of AD.[14] In the autopsy population of the Honolulu-Asia Aging Study, the occurrence of neurofibrillary tangles and amyloid plaques in the hippocampus and cortex in people without the apolipoprotein E (*APOE*) ε4 allele were similar to those with and without DM. However, as for *APOE* ε4 carriers, these lesions were more common in people with DM than in people without DM.[12] It was also reported that DM is related to generating atherosclerosis and cerebral infarction, but not directly to AD pathology in diabetic patients with dementia.[15,16] Autopsy findings are usually a mixture of many changes occurring during the living state, so the findings do not necessarily reflect the changes that are clinically relevant.

Interaction between medication for DM, especially the effect of insulin use, and AD neuropathology should be considered as well, as the population of insulin users showed a much higher risk of developing dementia in a cohort study.[17] Biessels *et al.* showed significantly fewer amyloid plaques in diabetic patients who received both insulin and oral antidiabetic medication, as compared with diabetic patients with other medication statuses or non-diabetic subjects. The effects of diabetes medication were specific to amyloid plaques, as the extent of neurofibrillary tangles pathology was not associated with diabetes medications.[18] However, these findings are derived from autopsies, and it is not certain if the same results can be gained from living human brains.

Several neuroimaging studies reported that DM is a risk factor for silent and symptomatic brain infarcts seen with magnetic resonance imaging (MRI),[19,20] and DM is also associated with cortical and subcortical atrophy.[21–23] As functional imaging, it is well known that reductions in regional cerebral glucose metabolic rate (CMRglu), as measured by fludeoxyglucose F 18 positron emission tomography (FDG-PET), are associated with increased AD risk and can be observed years before the onset of dementia.[24,25] Baker *et al.* reported that insulin resistance in persons with normal cognition and prediabetes or early diabetes without treatment is associated with reductions in CMRglu measured with FDG-PET.[26] However, previous radiological studies had limitations on discussing the pathological mechanism, as the modalities used were not directly linked to Alzheimer's pathology. No studies have been carried out regarding a pathobiological link between DM and AD in living human subjects.

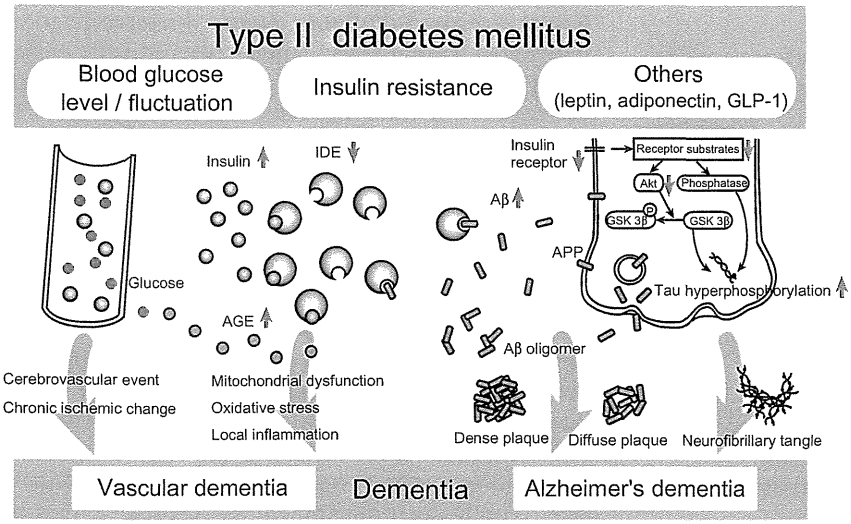

Figure 1 The possible pathological mechanisms associated with the impact of type 2 diabetes mellitus (DM) on the central nervous system (CNS). The major components of DM are described in the second column (only the three major components are described for easier understanding, though several other components are mentioned). Just below the column, the possible mechanism of developing dementia in type 2 DM. Aβ, amyloid β protein; AGE, advanced glycation end-products, APP, amyloid precursor protein; GLP-1, glucagon like peptide-1; GSK-3β, glycogen synthase kinase 3β; IDE, insulin degrading enzymes.

Table 1 Demographic data of the study participants

	Diagnostic group Normal control	AD alone	AD with DM
n	14	11	4
Sex (male/female)	7/7	4/7	2/2
Age	64.5 ± 2.9	78.5 ± 3.9	77.5 ± 5.2
MMSE	29.9 ± 0.1	20.5 ± 0.8	19.4 ± 2.8
ApoE ε4 allele (%)	0.12	0.35	0.37
HbA_{1c} (%)	5.7 ± 0.1	5.8 ± 0.1	7.2 ± 0.4

AD, Alzheimer's disease; DM, diabetes mellitus; HbA_{1c}, glycated hemoglobin; MMSE, Mini-Mental State Examination.

In order to clarify etiology and dementia subtypes in diabetic patients, we took a unique approach to visualize amyloid β protein (Aβ) deposition by positron emission tomography (PET) in living diabetic patients with dementia. The Aβ accumulation is successfully and non-invasively visualized by a recently-developed novel amyloid imaging probe called BF-227.[27–31] We used this tracer and applied it to "diabetic" and "non-diabetic" patients with clinically-diagnosed AD, to obtain more insights into differences in the extent and distribution of Aβ accumulation between diabetic and non-diabetic groups.

Methods

A total of 14 normal controls (NC), four diabetic patients with AD (AD with DM) and 11 non-diabetic patients with AD (AD alone) were examined. All the dementia patients were clinically diagnosed as probable AD according to the clinical criteria by "the National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association".[32] Brain MRI (1.5 Tesla; General Electric, Fairfield, CT, USA) was carried out on all the participants to exclude other causes of dementia. All the DM types of diabetic patients with AD were type 2. 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.

The PET procedure using BF-227 is described elsewhere.[28,31] BF-227 and its *N*-desmethylated derivative (a precursor of [^{11}C]BF-227) were custom-synthesized by Tanabe R&D Service (Osaka, Japan) [^{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 carried out using a PET SET-2400W scanner (Shimadzu, Kyoto, 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 bodyweight. Regions of interest (ROI) were placed on individual axial 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 SUV were sampled using Dr.View/LINUX software (AJS, Tokyo, Japan). Because there were neither senile plaques nor glucose hypometabolism in the cerebellum of AD patients, the ratios of regional SUV to cerebellar SUV (SUVR) were calculated as an index of [^{11}C]BF-227 retention. Neocortical SUVR was calculated by averaging SUVR in the frontal, lateral temporal, parietal and posterior cingulate cortices. Apolipoprotein E genotyping was carried out as previously described.[33]

The difference of Neocortex SUVR between the group of AD with DM and other groups was assessed with Student's *t*-test. The performance of diagnostic indices to discriminate among groups was assessed using receiver operating characteristic (ROC) analysis. Areas under ROC curves (AUC) were calculated and compared using GraphPad Prism Software (GraphPad Software, San Diego, CA, USA). Statistical significance was defined as $P < 0.05$.

Results

The clinical features of the three groups, NC, AD alone and AD with DM, are described in Table 1. Severities of dementia assessed by Mini-Mental State Examination were not significantly different between AD alone and AD with DM. Three patients were treated with only oral DM medications (patient A glimepiride + pioglitazone; patient B glimepiride + metformin + voglibose; patient

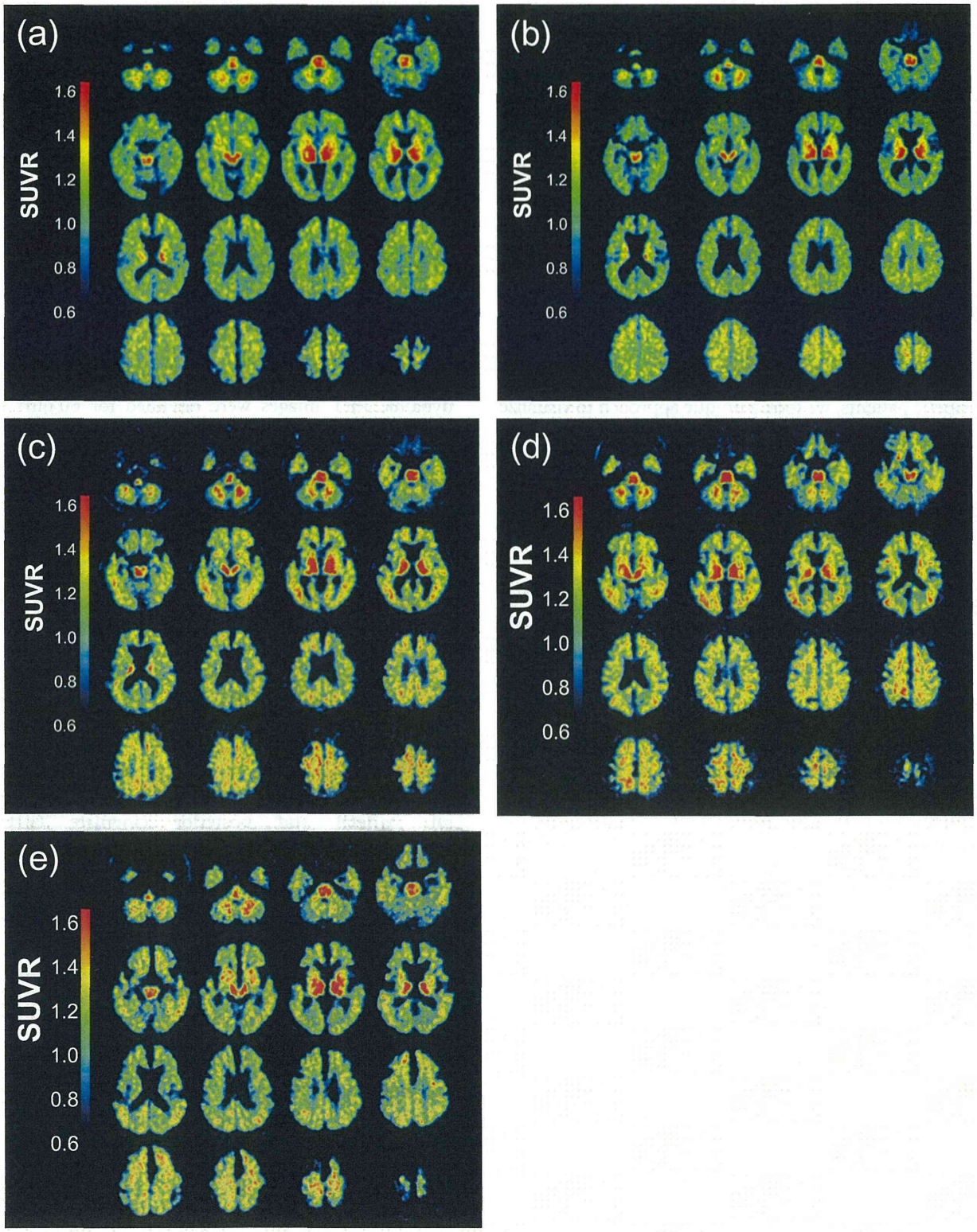

Figure 2 Representative BF-227 positron emission tomography images of each diagnostic group. (a) Normal control without diabetes mellitus (DM; 67-years-old, male, no complication; neocortical ratios of regional standardized uptake value to cerebellar standard uptake value ratio [SUVR] = 1.122). (b) Normal control with diabetes mellitus (67-years-old, female, insulin user; neocortical SUVR = 1.012). (c) Alzheimer's disease (AD) alone (75-years-old, female; neocortical SUVR = 1.230). (d) AD with DM (79-years-old, female, insulin user; neocortical SUVR = 1.240). (e) AD with DM (78-years-old, male, non-insulin user; SUVR = 1.18).

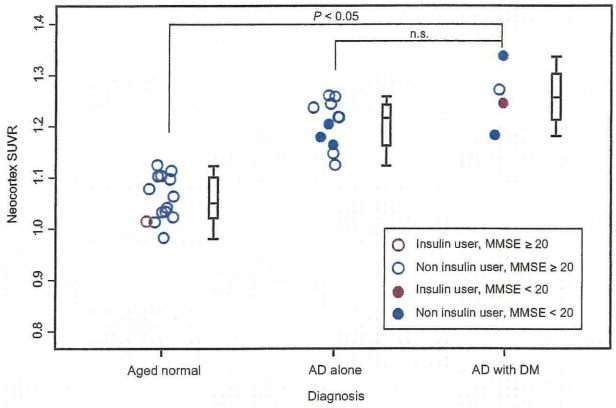

Figure 3 Box and scatter plots of ratios of regional standardized uptake value to cerebellar standard uptake value ration (SUVR) values with BF-227 in aged normal, Alzheimer's disease (AD) alone and AD with diabetes mellitus (DM) participants. Each circle indicates the mean SUVR from the mean neocortex. Red colored circle represents insulin user, whereas blue colored circle represents non-insulin user. There are no DM patients in the aged normal group shown with the blue circle. The filled circle represents the participants with Mini-Mental State Examination score less than 20. Although both AD with DM and AD alone showed significantly higher SUVR than the normal control group ($P < 0.05$), the difference between AD with DM and AD alone was not significant (n.s.).

Table 2 Characteristics of insulin users

	Subject 1 (no. 4) (normal cognition)	Subject 2 (no. 6) (AD patients)
Age	67	79
Sex	Female	Female
MMSE	28	21
ApoE genotype	3/3	3/3
CSF total tau (pg/ml)	–	334
BMI	24.7	19.8
HbA_{1c} (%)	7.6	8.2
Medication	Insulin only	Insulin, metformin
Hypoglycemic event	several	none
Duration of insulin use (years)	11	7

AD, Alzheimer's disease; BMI, body mass index; CSF, cerebrospinal fluid; HbA_{1c}, glycated hemoglobin; MMSE, Mini-Mental State Examination.

C metformin + voglibose), whereas only one AD with DM patient used insulin in addition to metformin. One DM patient was present in the normal control group. This patient in the control group had no oral medication. Insulin injection was the only medication.

MRI scans showed no or very few ischemic or hemorrhagic lesions observed in any of the participants. These small lesions were not strategic. White matter lesions (both periventricular and deep white matter) are all less than mild according to the Fazekas criteria (data not shown).[34]

After we obtained demographic information, we analyzed PET images with BF-227 among the three groups, and representative brain PET images are shown in Figure 2. As indicated in the figure, both the patients with AD alone and AD with DM showed significantly more robust retention of BF-227 than NC. Statistical analysis showed a significantly higher SUV-R of BF-227 ($P < 0.05$) in the cerebral cortex of AD alone and AD with DM than NC, as shown in Figure 3. Neocortical SUV-R of BF-227 in AD alone and AD with DM are not significantly different. Both the patients with AD alone and AD with DM showed increased BF-227 uptake in frontal, temporal, parietal, occipital and cingulated gyrus. The pattern of uptake was similar between the DM patients with insulin use and those without the use of insulin (Fig. 2). A similar pattern of uptake between insulin users and non-insulin users was seen both in the control group and the AD with DM group.

The clinical profiles of the two insulin users are shown in Table 2.

Discussion

The present study had two major findings. First, the uptake of BF-227 was significantly higher in both AD groups than that of the normal control group, regardless of DM complication. Second, the amount and pattern of the uptake was not affected by the use of insulin, both in the control group and the AD with DM group.

The first result that the severity and extent of the deposition did not differ significantly between the two groups suggests that both AD with DM and AD alone have robust deposition of senile plaques or typical AD pathology. In addition, all the participants we examined showed no or very few vascular lesions observed with MRI, indicating that we could exclude vascular dementia. The present result showed that the cause of developing dementia in DM patients cannot be fully explained by vascular mechanism. From the results of previous studies,[13,14] we assumed that either extra or less deposition of amyloid plaques would be seen in the brain of AD patients with DM complication. However, the brains of AD patients with DM showed a similar pattern and severity of the amyloid deposition to that seen in the brains of AD without DM complication. One possible explanation is that some kinds of protein that cannot be detected by BF-227 play a more important role than the classical aggregated plaque. Soluble

Aβ oligomers, which cannot be detected by BF-227, were shown to lower insulin receptor responses to insulin and cause substantial loss of neuronal surface insulin receptors.[35] Another possibility is that the additional effect of DM complication appears mainly through the increase in phosphorylation of tau, instead of an increase of Aβ plaque.

The second result of AD patients is in conflict with those reported by Beeri *et al.*[18] According to their conclusion, the AD patient with insulin and metformin use (subject 4 in Fig. 2) should have shown fewer senile plaques (lower uptake) as compared with diabetic patients with other medication status or non-diabetic subjects. One explanation for this inconsistency is that he/she was an *APOE* ε4 non-carrier. The occurrence of neurofibrillary tangles and amyloid plaques in people without the *APOE* ε4 allele were similar to those with and without DM in the autopsy population of the Honolulu-Asia Aging Study.[12] It is assumed that the effect of insulin and other medication use on reducing the plaques might only be effective in reducing the extra deposition of amyloid plaques in *APOE* ε4 carriers.

It was also found that the insulin user with normal cognition (subject 2 in Fig. 2) showed no difference in uptake. This subject was not obese, and started insulin injections 11 years before she undertook the PET procedure. Her glycohemoglobin level was 7.6%, and she had experienced several hypoglycemic events just before participation in the present study. From these clinical features, we assume that one of the main components of her DM were fluctuations of her blood glucose level (hyperglycemia and hypoglycemia). The interaction with ApoE ε4 might also be thought to be an explanation.

A limitation of the present study was that we could not adjust some factors, such as age, due to the small sample size. Because of the small sample size, the present study should be treated as a preliminary report. In addition, we could not measure the value of their homeostasis model assessment ratio, which is one of the key indicators of insulin resistance. We could not measure this indicator of insulin users, because they already had started insulin before admission to our clinic. Further studies are required to clarify the present report.

In conclusion, the present study provided new and important preliminary findings that a similar pathomechanism, which is the deposition of robust aggregated Aβ in the brain, is shared in both AD with DM and AD alone.

Disclosure statement

The authors declare no conflict of interest.

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