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Preserved benzodiazepine receptors in Alzheimer's disease measured with C-11 flumazenil PET and I-123 iomazenil SPECT in comparison with CBF

Masashi OHYAMA,*,** Michio SENDA,** Kiichi ISHIWATA,** Shin KITAMURA,* Masahiro MISHINA,* Kenji ISHII,** Hinako TOYAMA,** Keiichi ODA** and Yasuo KATAYAMA*

*Second Department of Internal Medicine, Nippon Medical School
**Positron Medical Center, Tokyo Metropolitan Institute of Gerontology

This study evaluates the regional cerebral blood flow (CBF) with $H_2{}^{15}O$-PET and the distribution of central benzodiazepine receptor (BZR) with C-11 flumazenil (FMZ) by PET and I-123 iomazenil (IMZ) by SPECT in Alzheimer's disease (AD). In AD, whereas the CBF was diminished in the frontal, temporal, parietal, and occipital cortex, the distribution volume of FMZ and delayed activity of IMZ were relatively preserved in these cortices, suggesting that the BZR reduction, reflecting neuronal loss, is less prominent than the CBF suppression. The mini-mental state examination score (MMS) was weakly correlated with the CBF in the parietal cortex but not with BZR. It is speculated that the neuronal density reflected by BZR is less impaired than the neuronal function assessed with blood flow in the association cortex of AD.

High correlation was found between the uptake of FMZ and the delayed activity of IMZ. The delayed image of IMZ-SPECT is clinically useful to evaluate the preservation of neuronal density in the affected temoporoparietal association cortex in AD.

Key words: C-11-flumazenil, I-123-iomazenil, positoron emission tomography, cerebral blood flow, Alzheimer's disease

INTRODUCTION

ALTHOUGH ALZHEIMER'S DISEASE (AD) is characterized by a decrease in blood flow and glucose metabolism in the temporoparietal association cortex, not much is known about the γ-aminobutyric acid A ($GABA_A$) receptor in AD. Because $GABA_A$ receptor is amply distributed on inter-neurons throughout the cerebral cortex, its evaluation reveals the neuronal integrity, an indicator of the disease severity different from blood flow or metabolism.[1–12] Recently, radiopharmaceuticals for imaging central benzodiazepine receptor (BZR), which is closely related to $GABA_A$ receptor, have been introduced to human use: ^{11}C-flumazenil (FMZ) for PET[13–17] and ^{123}I-iomazenil (IMZ) for SPECT.[18–23]

Results of previous investigations on $GABA_A$ or BZR in AD are inconsistent. Some *in vitro* studies reported a decrease in BZR in the frontal and temporal cortex[24] as well as in the hippocampus,[25] but others showed no significant reduction in any cerebral cortical areas.[26,27] In the living human brain, Meyer and colleagues[17] used FMZ with PET and reported that association cortical benzodiazepine binding sites in patients with AD were relatively well preserved, suggesting structurally intact cortical neuropil underlying glucose hypometabolism. Kitamura[23] and colleagues reported that there was no significant difference in delayed IMZ uptake (ratio to cerebellum) between patients with AD and the controls, suggesting that benzodiazepine binding sites are relatively well preserved in early AD. Other investigators reported a more prominent reduction in IMZ uptake than CBF decrease measured by HMPAO in patients with AD.[28] As cognition enhancers in relation to benzodiazepine receptors have been proposed as a treatment of

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For reprint contact: Masashi Ohyama, M.D., Positron Medical Center, Tokyo Metropolitan Institute of Gerontology, 1–1 Naka-cho, Itabashi-ku, Tokyo 173–0022, JAPAN.
E-mail: m.oyama@nms.ac.jp.

AD,[29–32] *in vivo* evaluation of BZR is becoming more important.

Although both FMZ and IMZ seem suitable as a marker of BZR,[33] few studies compared the two tracers on the same set of subjects. FMZ is a benzodiazepine antagonist,[16] but IMZ is a partial inverse agonist.[19] There is the possibility of different regional distribution of the two tracers.

One of the aims of the present investigation is to evaluate the loss of BZR with FMZ and to compare it with CBF measured with O-15 water by PET in AD patients. Second, we compare the distribution of FMZ by PET with that of IMZ by SPECT on the same patients with AD. We examine the clinical usefulness of the delayed uptake images of IMZ in comparison with the distribution of FMZ by using PET.

MATERIALS AND METHODS

Subjects

Five patients (age 68.0 ± 7.0 y.o.; range 59–76 y.o., all females) clinically diagnosed as probable AD by the criteria of the NINCDS-ADRDA[34] underwent PET scannings with FMZ and $H_2{}^{15}O$ and a SPECT scanning with IMZ. The mini-mental status (MMS)[35] scores at the times of the SPECT and PET examinations and the functional assessment staging of SDAT (FAST) scores[36] were determined at the time of $H_2{}^{15}O$ and FMZ-PET. The intervals between the IMZ-SPECT and FMZ-PET were 9.8 ± 1.5 months. The clinical profile of the patients is given in Table 1. MRIs were taken of each patient to obtain morphological information. Five normal elderly control subjects (age 61.2 ± 6.22 y.o.; range 57–72 y.o., all females) underwent PET scannings with FMZ and $H_2{}^{15}O$. Human use of the PET radiopharmaceuticals has been approved by the Radiopharmaceutical Committee, and the study protocol was approved by the Ethics Committee of Tokyo Metropolitan Institute of Gerontology and of Nippon Medical School. Written informed consent was obtained from each normal subject and from each patient.

PET imaging

A PET scanner Headtome IV (Shimadzu)[37] was used to measure CBF and BZR on the same day. The spatial and axial resolution was 7.5 mm and 9.5 mm FWHM (full-width at half-maximum), respectively. This system simultaneously acquires fourteen parallel slices with a center-to-center interslice distance of 6.5 mm.

CBF

The CBF was measured with an intravenous slow bolus injection of 1.5 GBq of O-15 water and the PET-autoradiographic method.[38] The data accumulation continued for 120 sec starting at the injection of O-15 water. The arterial blood activity was continuously measured and the CBF images were created.

FMZ

A dynamic imaging was performed for 60 minutes starting at the intravenous administration of 500 MBq of C-11 FMZ. The time course of the arterial plasma activity concentration, after correction for metabolites,[15] was used as the input function. With a two-parameter compartment model, parametric images were generated on a pixel-by-pixel basis, representing the ligand transport from blood to brain (K_1), washout (k_2), and the K_1/k_2 ratio as the distribution volume (DV) proportional to the binding site density.[14,16,39] Apart from it, the radioactivity images accumulated for 20 min starting 20 min post injection were created as the FMZ uptake image.

IMZ-SPECT imaging

An IMZ imaging was performed for 15 minutes starting at 172 minutes (mid-scan time: 180 minutes) after intravenous administration of 167 MBq of I-123 IMZ. A ring-type single photon emission computed tomograph (Headtome SET-080, Shimadzu Co.) was used to measure the distribution of radioactivity in the brain. The scanner simultaneously produces 32 tomographic axial slices. Data were recorded in a 128 × 128 matrix. A Butterworth and Ramp filter was used for image reconstruction. Reconstructed images were corrected for tissue absorption. The spatial and axial resolution was 8.5 mm and 17.5 mm FWHM, respectively. Before the study, the patients were given oral potassium iodide to prevent thyroid uptake of I-123. The delayed IMZ activity images obtained around 180 minutes after injection were used for the analysis of BZR distribution.[18,40]

Image analysis

The PET and SPECT data were analyzed with an image processor, Dr View (Asahi Kasei Joho System Co. Ltd., Tokyo, Japan) on Indigo2/Indy (Silicon Graphics Inc., CA, USA) computers. The IMZ SPECT images were registered to the subject's K_1 of FMZ-PET three-dimensionally with the software by Senda.[41] On the CBF images of each subject, ten circular regions of interest (ROIs) 12 mm in diameter were drawn over the frontal, temporal, parietal, occipital and cerebellum in both hemispheres in each subject to obtain the regional value of CBF, FMZ-K_1 and FMZ-DV for each ROI. As a simplified analysis, the ratio of each cerebral cortical ROI to the cerebellum was also computed for the FMZ uptake image (acquired from 20 to 40 min) and for the IMZ delayed activity image (acquired around 180 min) as proposed in previous reports.[40]

For the statistical analysis, ANOVA with Bonferroni's correction was employed with JMP (SAS Institute Inc., NC, USA), and Spearman rank correlation was employed with Stat View (Abacus Concepts, Inc., CA, USA) on a Macintosh personal computer.

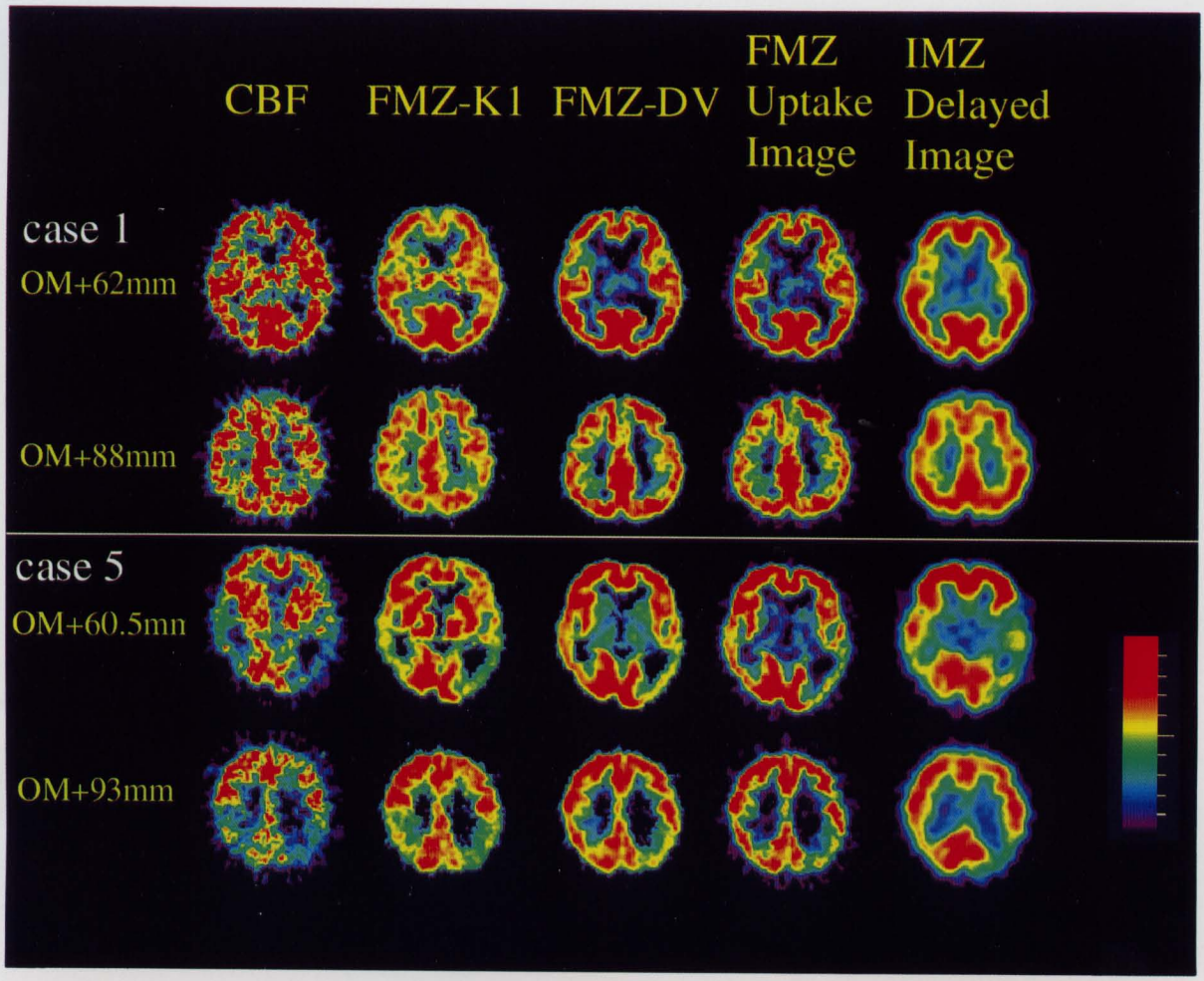

Fig. 1 PET images of CBF, FMZ-K_1, FMZ-DV (distribution volume), FMZ-uptake (from 20 to 40 min post injection) and SPECT image of IMZ delayed activity in Alzheimer patient case 1 (mild) and case 5 (moderate). Color scale is arbitrary.

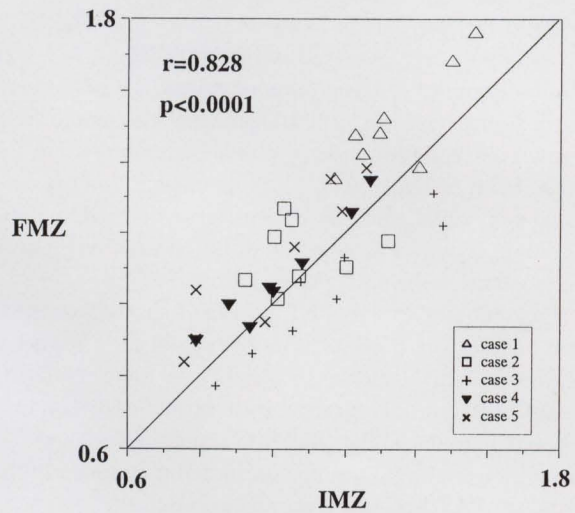

Fig. 2 Correlation of ROI values between FMZ uptake (from 20 to 40 min post injection) and IMZ delayed activity (at 180 min post injection). Both FMZ and IMZ are expressed by the ratio to cerebellum. Different symbols represent different patients.

RESULTS

Figure 1 illustrates the PET images of the CBF, FMZ-K_1, FMZ-DV, FMZ-uptake and SPECT images of IMZ delayed activity. The upper half shows images of case 1, a 67 y.o. female with mild dementia (FAST 3, MMS score 22/30), at OM + 62 mm and OM + 88 mm. The CBF and FMZ-K_1 were decreased in the parietal cortex but FMZ-DV, IMZ delayed activity, and FMZ uptake were preserved.

The lower half of Figure 1 shows images of case 5, a 59 y.o. female with moderate to severe dementia (FAST 5, MMS score 3/30) at OM + 60.5 mm and OM + 93 mm. Although all images showed a decrease in the temporoparietal cortex, FMZ-DV, IMZ delayed activity and FMZ uptake were less affected than CBF and FMZ-K_1. BZR in AD was therefore better preserved than CBF in the temporoparietal cortex.

Table 1 Clinical profile of patients with Alzheimer's disease

Patient's ID	Age	Sex	FAST	MMS (at the SPECT)	MMS (at the PET)	IMZ-FMZ interval (month)	Atrophy (MRI)	Duration of disease at the PET (year)
case 1	67	F	3	24	22	9.8	none	2
case 2	76	F	4	22	19	9.8	mild	3
case 3	64	F	4	13	11	8.2	mild	3
case 4	74	F	4	3	3	11.8	mild	4
case 5	59	F	5	3	3	8.1	mild	3

FAST: Functional Assessment Staging of SDAT
MMS: Mini-mental State Examination
IMZ-FMZ interval: The interval between the IMZ and FMZ studies

Table 2 CBF, FMZ-K_1 and FMZ-DV in normal elderly and Alzheimer's disease

CBF (ml/min/100 ml)		
Brain region	Normal elderly	AD
Frontal cortex	68.41 ± 9.23	45.43 ± 6.39*
Temporal cortex	73.20 ± 15.04	41.76 ± 4.99***
Parietal cortex	70.32 ± 14.88	35.84 ± 4.70****
Visual cortex	80.74 ± 21.80	56.78 ± 6.49*
Flumazenil K_1 (ml/min/ml)		
Brain region	Normal elderly	AD
Frontal cortex	0.31 ± 0.02	0.23 ± 0.02**
Temporal cortex	0.33 ± 0.03	0.22 ± 0.03*****
Parietal cortex	0.32 ± 0.04	0.20 ± 0.05*****
Visual cortex	0.37 ± 0.05	0.32 ± 0.06
Flumazenil DV (ml/ml)		
Brain region	Normal elderly	AD
Frontal cortex	4.59 ± 0.75	4.21 ± 1.65
Temporal cortex	4.84 ± 0.63	3.89 ± 1.61
Parietal cortex	4.60 ± 0.53	3.73 ± 1.58
Visual cortex	5.36 ± 0.57	5.05 ± 1.67

Values are Mean ± S.D. *****: $p < 0.0001$, ****: $p < 0.0005$, ***: $p < 0.001$, **: $p < 0.005$, *: $p < 0.05$ compared with normal elderly.

Comparison of CBF and distribution of benzodiazepine receptor

The result of ROI analysis is shown in Table 2. The CBF in the AD was significantly reduced in the frontal, temporal, parietal and occipital cortex compared with normal subjects. The FMZ-K_1 in the AD was significantly lower in the frontal, temporal, and parietal cortex than in normal elderly subjects. The FMZ-DV in the AD patients was not significantly different from normals in any region.

A high correlation was found between FMZ-DV and the FMZ uptake in pixel-by-pixel analysis (case 1: $r = 0.992$, case 2: $r = 0.789$, case 3: $r = 0.985$, case 4: $r = 0.981$, case 5: $r = 0.983$).

FMZ PET and IMZ SPECT

The reduction in the MMS score was within 3 points in AD patients during the SPECT and PET examinations (Table 1).

Significant correlation was found between the FMZ uptake (ratio to cerebellum) and the delayed activity of IMZ (ratio to cerebellum) by ROI analysis ($r = 0.828$) (Fig. 2). The plots were scattered around the line of identity dependent on the subject to some degree.

A weak correlation was found between the CBF and MMS scores in the parietal cortex (Spearman rank correlation: $\rho = 0.975$, $p = 0.051$), but no correlation was observed between the FMZ-DV and MMS scores (Spearman rank correlation: $\rho = 0.616$, $p = 0.218$).

DISCUSSION

Benzodiazepine receptor in Alzheimer

The present study on AD patients in the 3–5 FAST stages indicated that FMZ-DV, representing the binding capacity of BZR, was not significantly different from the normal elderly in the temporal, parietal or frontal cortex, although slightly lower than normal in average. On the other hand, CBF and FMZ-K_1 were significantly decreased in those areas in the AD patients. The BZR binding capacity assessed with FMZ-DV in AD was relatively preserved as compared with CBF in the temporoparietal cortex in each subject. Considering the well-known marked reduction in glucose metabolism in the association cortex of AD,[17] it is speculated that the neuronal density reflected by BZR is less impaired than the neuronal function assessed with blood flow or glucose metabolism in the association cortex of AD.

Previous studies with PET or SPECT showed inconsistent results for BZR in AD. Meyer et al.[17] reported relatively well preserved FMZ-DV in the association cortex of AD, mildly to severely demented AD when they merged 3 groups with mean MMS scores of 8, 12 and 22). Kitamura et al.[23] found no significant difference in the delayed IMZ activity (ratio to cerebellum) or in the washout (late-to-early) ratio between AD (FAST stages 3 to 5, MMS: 14.6 ± 2.12) and control. Fukuchi et al.[28] reported that the IMZ delayed activity ratio of the parietal lobe to cerebellum in AD (MMS score 20.4 ± 0.7) was

lower than that in the control group, and the affected areas depicted by IMZ-SPECT were more extensive than those of decreased CBF measured with HMPAO in all patients on visual inspection. Soricelli et al.[42] measured DV of IMZ in six patients with probable AD (MMS score 12.3 ± 1.41) and in five age-matched controls and found a reduced DV in all regions except the occipital cortex, in particular in the temporal and parietal cortices with statistical significance.

The inconsistent results may be caused by the difference in the tracer (FMZ or IMZ) methods (ratio or DV) or choice of reference (blood flow or cerebellum) as well as by the difference in the disease stage of the subjects.

When the cognitive function of the patients was assessed, MMS was correlated with CBF, not with FMZ, in the parietal cortex. This suggests that the reduction in CBF is related to neuronal dysfunction rather than to loss of neuronal density reflected in BZR in AD.

Some cognition enhancers are proposed as a treatment for AD in relation to benzodiazepine receptors.[29–32] As BZR and neurons measured by FMZ or IMZ are preserved in the early stages of AD, the patients may be treatable with those cognition enhancers having benzodiazepine inverse agonist-like properties.[29–31] It is also suggested that benzodiazepines may have protective effects against AD based on epidemiological evidence of an association between benzodiazepine use and the occurrence of AD and vascular dementia in 668 individuals.[43] The *in vivo* measurement of density of BZR may provide the indication of the drug medication for AD patients.

FMZ-PET and IMZ-SPECT

IMZ is a SPECT ligand with a high selectivity for BZR and an affinity ten-fold greater than that of FMZ.[18–22] Although its kinetics has not been studied in detail and the method of quantitative analysis has not been established,[18] delayed images acquired 180 minutes post injection are reported to represent BZR binding capacity.[40] Therefore, considering that the neurons of the cerebellum are not involved in AD patients, the region-to-cerebellar ratio of the delayed images was used as a measure of BZR in the present study according to the methods in previous reports.[23,28]

The uptake of FMZ measured between 20 min and 40 min post injection is known to be approximately proportional to the DV of FMZ and therefore to BZR binding capacity.[44] In the present study as well, the FMZ uptake had a high correlation with FMZ-DV in pixel-by-pixel analysis within subjects (r = 0.789 to 0.992). We therefore examined the correlation of the region-to-cerebellar ratio between the IMZ delayed image and FMZ uptake. The result indicated a high and significantly positive correlation between IMZ and FMZ (Fig. 2). This is surprising because the physical image quality, including resolution, attenuation and scatter, in PET and SPECT is quite different and a positioning error may have occurred in the course of image registration. The close correlation in Figure 2 suggests that both IMZ and FMZ are equally representative of BZR in AD. The reduction in the MMS score was within 3 points in AD patients during the SPECT and PET examinations (Table 1), suggesting there was no great change in the cognitive function clinically in the duration. This is also supported by the fact that the plots were scattered around the line of identity without an obvious offset. Slight individual difference in their relationship may be attributed to the individual difference in the input function. Because IMZ is more readily available than FMZ when it comes on the market, the results suggest that delayed activity of IMZ-SPECT is clinically useful to evaluate the preservation of neuronal density in the affected temporoparietal association cortex of AD patients.

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