Peripheral benzodiazepine receptors in patients with chronic schizophrenia: a PET study with $^{[11C]}$DAA1106

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Abstract

Inflammatory/immunological process and glial contribution are suggested in the pathophysiology of schizophrenia. We investigated peripheral benzodiazepine receptors in brains of patients with chronic schizophrenia, which were reported to be located on mitochondria of glial cells, using $^{[11C]}$DAA1106 with positron emission tomography. Fourteen patients and 14 age- and sex-matched normal controls participated in this study. PET data were analysed by two-tissue compartment model with metabolite-corrected plasma input. Clinical symptoms were assessed using the Positive and Negative Syndrome Scale. There was no significant difference between $^{[11C]}$DAA1106 binding of the cortical regions of normal controls and patients with schizophrenia, whereas the patients showed a positive correlation between cortical $^{[11C]}$DAA1106 binding and positive symptom scores. There was also a positive correlation between $^{[11C]}$DAA1106 binding and duration of illness. Although the correlations need to be interpreted very cautiously, involvement of glial reaction process in the pathophysiology of positive symptoms or progressive change of schizophrenia might be suggested.

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Key words: Microglia, peripheral benzodiazepine receptor, positive symptoms, schizophrenia.

Introduction

An accumulating body of evidence has suggested that the pathophysiology of schizophrenia could be related to the dysregulation of the inflammatory response system, such as increased levels of in vivo IL-1RA, sIL-2R, and IL-6 (Lin et al. 1998; Nawa & Takei, 2006; Potvin et al. 2008; Zhang et al. 2004). Microglia has been regarded as a mediator of neuroinflammation via the release of pro-inflammatory cytokines, nitric oxide (NO) and reactive oxygen species (ROS) in the central nervous system (CNS). Peripheral benzodiazepine receptor (PBR) was reported to reflect neuronal injury and inflammatory lesions in the brain by increased expression of the number of binding sites in glial cells including activated microglia and reactive astrocytes as visualized in vivo using PET with $^{[11C]}$PK11195 (Shah et al. 1994). Recent reports demonstrated that $^{[11C]}$PK11195 binding was increased in patients with acute-onset schizophrenia (van Berckel et al. 2008) and in patients with schizophrenia during psychosis (Doorduin et al. 2009). However, the affinity (Chaki et al. 1999) and permeability of the blood–brain barrier was low for PK11195, reportedly a substrate of efflux transporter P-glycoprotein (Jakubikova et al. 2002; Vaalburg et al. 2005). Low uptake of $^{[11C]}$PK11195 in the brain could hamper stable quantitative analysis.

(N-5-fluoro-2-phenoxyphenyl)-N-(2,5-dimethoxybenzyl) acetamide (DAA1106) is a potent and selective ligand for PBR with high affinity (Chaki et al. 1999; Okuyama et al. 1999). $^{[11C]}$DAA1106 is accumulated at high levels in the mouse brain (Zhang et al. 2003), and the radioactivity of $^{[11C]}$DAA1106 at 30 min after injection was reported to be four times higher than that of $^{[11C]}$PK11195 in the monkey brain (Maeda et al. 2004). A quantitative analysis method for $^{[11C]}$DAA1106 binding in the human brain has been well established with the two-tissue compartment model (Ikoma et al. 2007). $^{[11C]}$DAA1106 was
demonstrated to be useful in the study of neurodegenerative disorders such as Alzheimer’s disease (Yasuno et al. 2008).

In this study, we investigated PBR binding in patients with chronic schizophrenia using [11C]DAA1106 to evaluate whether glial reaction was involved in the pathophysiology of schizophrenia.

Materials and methods

Subjects

Fourteen patients with schizophrenia [six females, eight males; 43.9 ± 7.4 yr (mean ± s.d.)] and 14 normal control subjects [five females, nine males; 42.5 ± 9.0 yr] were enrolled in this study. Patients were recruited from the outpatient and in-patient units of Nippon Medical School Hospital, Asai Hospital and Sobu Hospital, located in Tokyo and Chiba prefecture in Japan. The patients were diagnosed as having schizophrenia and treated by attending physicians at each hospital, and their diagnoses were re-evaluated with structured interviews at our PET centre. All 14 patients were diagnosed with schizophrenia according to DSM-IV criteria. Exclusion criteria were current or past substance, cannabis or alcohol abuse, mood disorders, and organic brain disease. The patients’ demographic and clinical data are shown in Table 1. None of the patients had taken benzodiazepines within more than 1 month prior to PET measurements.

Psychopathology was assessed by the Positive and Negative Syndrome Scale (PANSS; Kay et al. 1987). PANSS was completed by three experienced psychiatrists on the same day as the PET measurements. They reviewed the ratings after the interviews, and disagreements were resolved by consensus; the consensus ratings were used in this study. The symptom scores were calculated as total scores, positive symptom, negative symptom, and general symptom sub-scores of PANSS. The total PANSS score ranged from 49 to 117 (78.6 ± 20.7). The mean positive symptom score was 19.1 ± 5.3, negative symptom score was 22.1 ± 6.5, and general symptom score was 37.4 ± 11.1.

The normal control subjects were recruited from the surrounding community. Based on psychiatric screening interviews, they were free of current and past psychiatric or major medical disease, and had no relatives with neuropsychiatric disorders.

This study complied with the current laws of Japan, and was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiological Sciences, Chiba, Japan. Written informed consent was obtained from all subjects.

Radiochemistry

[11C]DAA1106 was prepared as described in detail previously (Ikoma et al. 2007; Zhang et al. 2003). The precursor was supplied by Taisho Pharmaceutical Co. (Japan).

Table 1. Demographic and clinical characteristics of the patients with schizophrenia

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yr), sex</th>
<th>PANSS</th>
<th>Duration of illness (yr)</th>
<th>Duration of drug treatment (yr)</th>
<th>Haloperidol equivalent (mg)</th>
<th>Main antipsychotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>General</td>
<td>Total</td>
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<td>1</td>
<td>29, F</td>
<td>12</td>
<td>12</td>
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<td>49</td>
<td>11</td>
</tr>
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<td>34, F</td>
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<td>12</td>
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<td>3</td>
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<tr>
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<td>49</td>
<td>97</td>
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</tr>
<tr>
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<td>46, F</td>
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<td>15</td>
<td>34</td>
<td>65</td>
<td>33</td>
</tr>
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<td>49, F</td>
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<td>20</td>
<td>33</td>
<td>77</td>
<td>23</td>
</tr>
<tr>
<td>7</td>
<td>42, M</td>
<td>15</td>
<td>22</td>
<td>27</td>
<td>64</td>
<td>4</td>
</tr>
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<tr>
<td>9</td>
<td>44, M</td>
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<td>25</td>
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<td>87</td>
<td>22</td>
</tr>
<tr>
<td>10</td>
<td>44, M</td>
<td>16</td>
<td>26</td>
<td>37</td>
<td>79</td>
<td>4</td>
</tr>
<tr>
<td>11</td>
<td>46, M</td>
<td>29</td>
<td>26</td>
<td>56</td>
<td>111</td>
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<tr>
<td>12</td>
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<td>16</td>
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<td>25</td>
<td>57</td>
<td>24</td>
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<tr>
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<td>35</td>
<td>58</td>
<td>117</td>
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<tr>
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<td>59, M</td>
<td>27</td>
<td>24</td>
<td>47</td>
<td>87</td>
<td>43</td>
</tr>
</tbody>
</table>

PANSS, Positive and Negative Syndrome Scale; F, female; M, male.

Haloperidol (1 mg) was equivalent to chlorpromazine (50 mg).
PET data acquisition

PET scans were performed with ECAT EXACT HR+ (CTI-Siemens, USA), which provides 63 planes and a 15.5-cm axial field of view (FOV). A 10-min transmission scan with a $^{68}$Ge-$^{68}$Ga source was followed by a 90-min dynamic scan (20s × 9, 60s × 5, 120s × 4, 240s × 11, and 300s × 6) with a bolus injection of 261–411 (369 ± 27) MBq of $[^{11}C]$DAA1106. Specific radioactivity was 15.4–220.7 GBq/mmol at the time of the injection. There was no significant difference in injected radioactivity and specific radioactivity between patients and normal controls (373 ± 20 MBq and 60.3 ± 44.4 GBq/mmol for patients, and 366 ± 32 MBq and 98.4 ± 70.7 GBq/mmol for normal controls). Radioactivity was measured in three-dimensional mode, and the data were reconstructed with a Hanning filter with a cut-off frequency of 0.4 (full width half maximum = 7.5 mm).

Arterial blood sampling

To obtain the arterial input function, an automated blood sampling system was used for continuous (counts/s) blood radioactivity measurements during the first 12 min of PET measurement. At the same time, arterial blood samples were taken manually and their radioactivity concentration was measured 13 times during the initial 3 min after the injection, eight times during the next 17 min, and once every 10 min until the end of the scan. To analyse the metabolite fraction in the plasma, arterial blood samples were taken 10 times during PET measurements. The parent ligand, separated from the total radioactive compound, was measured as previously described (Ikoma et al. 2007). The mean time-course of the fraction of the parent ligand is shown in Fig. 1. There was a significant group × time interaction using repeated-measures ANOVA with Greenhouse–Geisser correction ($F_{3.4,81} = 4.92$, $p = 0.002$), although one subject from each group was excluded for the statistical analysis due to one missing data-point.

MR imaging

T1-weighted magnetic resonance imaging (MRI) of the brain was performed with Philips Intera 1.5 T (Philips Medical Systems, The Netherlands). T1-weighted images of the brain were obtained from all subjects. The scan parameters were 1-mm-thick 3D T1 images with a transverse plane [repetition time (TR)/echo time (TE) 22/9.2 ms, flip angle 30°, matrix 128 × 128, FOV 256 × 256]. Voxel size of the magnetic resonance images was 1 mm × 1 mm × 1 mm.

Fig. 1. Mean time-course of the percentage of parent compound ($[^{11}C]$DAA1106) after venous injection of $[^{11}C]$DAA1106 between normal controls (–○–) and patients (–●–) with schizophrenia.

Data analysis

Eleven regions of interest (ROIs) (medial frontal cortex, dorsolateral frontal cortex, medial temporal cortex, lateral temporal cortex, parietal cortex, occipital cortex, thalamus, striatum, cerebellum, anterior cingulate cortex, and posterior cingulate cortex) were delineated on the co-registered PET/MRI images. In addition to each regional ROI, eight cortical ROIs (medial frontal cortex, dorsolateral frontal cortex, medial temporal cortex, lateral temporal cortex, parietal cortex, occipital cortex, anterior cingulate cortex, and posterior cingulate cortex) were also summed up as total cortical regions.

Regional time–activity data were analysed with two-tissue compartment model (2-TC) with the metabolite-corrected plasma input function, a model demonstrated to estimate binding potential (BP$_{ND}$) most reliably for $[^{11}C]$DAA1106 (Ikoma et al. 2007). Rate constants were estimated with weighted least squares and the Marquardt optimizer. For each region, $k_3$, $k_2$, $k_b$, $k_c$ and blood volume were estimated by 2-TC. BP$_{ND}$ was calculated as $k_b/k_4$ in this analysis. Data analysis was performed with PMOD 2.65 (PMOD Technologies, Switzerland).

Statistical analysis

Regional ROIs

Statistical analysis of the difference of regional BP$_{ND}$ for each ROI (for total 11 ROIs) between patients and normal controls was performed by repeated-measures ANOVA ($p < 0.05$ was considered significant). When any interaction was found, post-hoc Bonferroni correction was used for multiple comparisons.
Correlation between regional BP ND values and PANSS scores were analysed with Pearson’s correlation method (\(p < 0.05\) was considered significant).

Correlation between regional BP ND values and duration of illness, duration of drug treatment, and chlorpromazine equivalent doses (Inagaki et al. 1999) were analysed with Pearson’s correlation method (\(p < 0.05\) was considered significant).

Changes in regional BP ND values with age were analysed with Pearson’s correlation method for patients and normal controls, respectively (\(p < 0.05\) was considered significant).

Changes in BP ND values in total cortical regions with age were analysed with Pearson’s correlation method for patients and normal controls, respectively (\(p < 0.05\) was considered significant).

### Results

#### Regional ROIs

Comparison of regional BP ND values for \([^{11}C]DAA1106\) between the patients with schizophrenia and normal controls by two-way repeated ANOVA with Greenhouse–Geisser correction showed no significant group × region interaction (\(F_{15,44.4} = 0.542, p = 0.558\)).

For the correlation analysis between BP ND values in regional ROIs and positive symptom scores in the patient group, significant correlations were found in regions such as the medial frontal cortex, medial temporal cortex and occipital cortex (Table 2) (Fig. 2). No correlation was found between BP ND values of each region and negative symptoms. Those three regions showed trends of positive correlation with general symptoms and total score (Table 2). There was no significant correlation between regional BP ND and the duration of illness.

There was no significant change of regional BP ND values with age in normal controls, whereas significant changes in BP ND values with age in the patients with schizophrenia were observed in the occipital cortex (\(p = 0.014\)), lateral temporal cortex (\(p = 0.023\)), parietal cortex (\(p = 0.023\)), medial temporal cortex (\(p = 0.031\)), and medial frontal cortex (\(p = 0.036\)).

#### Total cortical regions

For analysing differences in total cortical regions between patients and normal controls, Student’s \(t\) test was used (\(p < 0.05\) was considered significant).

Correlations between BP ND values in total cortical regions and PANSS scores were analysed with Pearson’s correlation method (\(p < 0.05\) was considered significant).

**Table 2. Significant correlation between PANSS scores and regional \([^{11}C]DAA1106\) binding**

<table>
<thead>
<tr>
<th>PANSS scores</th>
<th>Region</th>
<th>(p) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive symptom</td>
<td>Medial frontal cortex</td>
<td>0.002*</td>
</tr>
<tr>
<td></td>
<td>Dorsolateral frontal cortex</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>Medial temporal cortex</td>
<td>0.003*</td>
</tr>
<tr>
<td></td>
<td>Lateral temporal cortex</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>Parietal cortex</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Occipital cortex</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>Cerebellum</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>Striatium</td>
<td>0.010</td>
</tr>
<tr>
<td>Negative symptom</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>General symptom</td>
<td>Medial frontal cortex</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>Medial temporal cortex</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td>Occipital cortex</td>
<td>0.038</td>
</tr>
<tr>
<td>Total score</td>
<td>Medial frontal cortex</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>Medial temporal cortex</td>
<td>0.029</td>
</tr>
<tr>
<td></td>
<td>Parietal cortex</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>Occipital cortex</td>
<td>0.017</td>
</tr>
</tbody>
</table>

PANSS, Positive and Negative Syndrome Scale.  
*\(p < 0.0045 (0.05/11)\).*

Fig. 2. Positive correlation between \([^{11}C]DAA1106\) BP ND in the occipital cortex and positive symptom scores in the Positive and Negative Syndrome Scale.
Total cortical regions

There was no significant difference of BP<sub>ND</sub> values in total cortical regions between patients with schizophrenia and normal controls (Fig. 3). Significant correlation was found with the positive symptom scores (p = 0.006) (Fig. 4). There was no significant correlation with other symptom scores (negative, general, and total symptom scores). Total cortical regions were correlated with duration of illness (p = 0.020) (Fig. 5) and duration of drug treatment (p = 0.023). BP<sub>ND</sub> of total cortical regions was not correlated with chlorpromazine-equivalent doses.

There was no significant change of BP<sub>ND</sub> values in total cortical regions with age in normal controls, but significant changes of BP<sub>ND</sub> values with age were observed in total cortical regions of the patients with schizophrenia (p = 0.018).

Discussion

In this study, [11C]DAA1106 binding, which was considered to correspond to the density of PBR, was not different between the patients with chronic schizophrenia and normal controls. A recent study demonstrated that [11C]PK11195 binding increased in total grey matter in patients with acute-onset schizophrenia (van Berckel et al. 2008). Another recent study reported that [11C]PK11195 binding in the hippocampus was significantly increased in patients with schizophrenia during acute psychosis, while there was no significant difference in other regions compared with normal controls (Doorduin et al. 2009). To understand the difference in the results between the present study and the two [11C]PK11195 studies, several factors, such as the use of different radioligands and different patient groups, should be taken into consideration. Although PK11195 fully displaced the [3H]DAA1106 binding (Chaki et al. 1999), a high concentration of PK11195 was required for this displacement. This suggested that the binding domain for DAA1106 contains an extra component that does not interact efficiently with PK11195 (Chaki et al. 1999). The mean age of patients with schizophrenia enrolled in the present study was higher (44 yr in 14 patients) than those in the two [11C]PK11195 studies (24 yr in 10 patients, and 31 yr in seven patients). Most of the patients in the present study were at the chronic stage.

Within the patient group, [11C]DAA1106 binding had a significant correlation with the positive symptom score of PANSS, a finding that might be in line...
with those recent findings with $^{11}$C]PK11195. The present results might indicate that the activated neuro-immune system was related to the pathophysiology of schizophrenia at the chronic stage.

In previous MRI volumetric research in schizophrenia, volume reduction in the brain has been reported in patients with chronic schizophrenia (Shenton et al. 2001). However, in the present study, there was no significant difference in the volume of ROIs by ANOVA, and total cortical ROI by Student’s t test between the patients and normal controls (data not shown). Thus, the insignificance of the difference of $^{11}$C]DAA1106 binding between the patients and normal controls is not related to the partial volume effect due to brain atrophy.

In this study, normal controls showed no age effects on $^{11}$C]DAA1106 binding in any region. This is in line with the report with $^{11}$C]PK11195 binding except the thalamus, where $^{11}$C]PK11195 binding was reported to increase with age (Cagnin et al. 2001). This might be due to different radioligands or different age ranges between the two studies (24–55 yr in this study and 32–80 yr in the $^{11}$C]PK11195 study). On the other hand, $^{11}$C]DAA1106 binding was found to increase with age in patients with schizophrenia. Schizophrenia has been considered to be progressive in functional disability and morphological changes (Lieberman et al. 2001; Mathalon et al. 2001; Saijo et al. 2001). The present results of the positive correlation among $^{11}$C]DAA1106 binding, duration of illness, and age might suggest that the progressive change occurs at the glial reaction level.

A recent meta-analysis showed that some cytokines such as IL-1RA, sIL-2R, and IL-6 are increased in schizophrenia (Potvin et al. 2008). PBR has been considered to modulate the release of pro-inflammatory cytokines in the CNS. PBR was reported to modulate the release of the inflammatory molecules NO and tumour necrosis factor-alpha (TNF-$\alpha$) (Wilms et al. 2003). A PBR ligand, PK11195, has been reported to inhibit lipopolysaccharide-induced expressions of COX-2 and TNF-$\alpha$ in human microglia (Choi et al. 2002). Immunomodulatory drugs such as cyclooxygenase-2 (COX-2) inhibitors have been reported to show beneficial effects in schizophrenia (Muller & Schwarz, 2008). The combination of risperidone and COX-2 inhibitor has been reported to show superiority over risperidone alone in positive symptoms and PANSS total scores (Akhondzadeh et al. 2007). On the other hand, cytokines such as IL-2 and IL-6 are reported to increase after olanzapine and clozapine treatment (Kluge et al. 2009). The present results of PBR binding in the patients with schizophrenia might be in accord with the previous reports of cytokines.

A recent report demonstrated that PBR expression was not confined to microglia but was inducible in nervous tissue cells of neuroepithelial origin (Ji et al. 2008). Thus, PBR binding might also arise from astrocytes and other non-microglial elements. Schizophrenia patients with high S100B serum concentration, considered to indicate astrocyte activation, were reported to have cognitive dysfunction compared with patients with low S100B serum concentration (Pedersen et al. 2008). DAA1106 binding in patients with schizophrenia might also be related to the change in PBR on astrocytes.

In a post-mortem study, a subgroup of the patients with schizophrenia who committed suicide had increased microglial densities, although microglial HLA-DR expression in the patients with schizophrenia was not different from normal controls (Steiner et al. 2008). Microglial activation has been suggested to be interpretable as a consequence of pre-suicidal stress (Avital et al. 2001; Lehmann et al. 2002).

Although BP ND of total cortical regions was not correlated with chlorpromazine-equivalent doses in the present study, some antipsychotics were reported to have anti-inflammatory effects (Kato et al. 2007; Kowalski et al. 2003, 2004; Labuzek et al. 2005; Zheng et al. 2008). The effect of antipsychotics on DAA1106 binding remains to be studied.

There are several confounding factors in the present study. First, the number of subjects was relatively small. Further larger-scale studies will be needed to confirm the present results. Second, all the patients were under different kinds of antipsychotic treatment. Further study is needed with drug-naive patients and patients under well-controlled drug treatment. Third, the PANSS scores of patients were higher as the duration of the illness was longer and age increased. This might reflect a possible subgroup of treatment-resistant patients.

In conclusion, we found no significant differences in PBR binding between the brains of patients with schizophrenia and those of normal control subjects, unlike recent reports with $^{11}$C]PK11195 (van Berckel et al. 2008; Doorduin et al. 2009). Nevertheless, PBR binding in the patients with schizophrenia was correlated with positive symptoms, disease duration and age. The present results suggest that the glial reaction process might be involved in the pathophysiology of schizophrenia. Although the correlations should be interpreted with caution, these results at least suggest that additional studies are warranted in order to determine whether baseline
differences exist between patients with schizophrenia and healthy subjects, as well as to reveal the biological meanings of the correlations with disease parameters.

Acknowledgments

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Statement of Interest

None.

References


