Neuroinflammation in Patients with Chronic Fatigue Syndrome/Myalgic Encephalomyelitis: An $^{11}$C-($R$)-PK11195 PET Study

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Chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) is a disease characterized by chronic, profound, disabling, and unexplained fatigue. Although it is hypothesized that brain inflammation is involved in the pathophysiology of CFS/ME, there is no direct evidence of neuroinflammation in patients with CFS/ME. Activation of microglia or astrocytes is related to neuroinflammation. $^{11}$C-($R$)-(2-chlorophenyl)(-methyl-N-methyl-(1-methylpropyl)-3-isoquinoline-carboxamide ($^{11}$C-($R$)-PK11195) is a ligand of PET for a translocator protein that is expressed by activated microglia or astrocytes. We used $^{11}$C-($R$)-PK11195 PET and PET to investigate the existence of neuroinflammation in CFS/ME patients.

Methods: Nine CFS/ME patients and 10 healthy controls underwent $^{11}$C-($R$)-PK11195 PET and completed questionnaires about fatigue, fatigue sensation, cognitive impairments, pain, and depression. To measure the density of translocator protein, non-displaceable binding potential ($BP_{ND}$) values were determined using linear graphical analysis with the cerebellum as a reference region.

Results: The $BP_{ND}$ values of $^{11}$C-($R$)-PK11195 in the cingulate cortex, hippocampus, amygdala, thalamus, midbrain, and pons were 45–199% higher in CFS/ME patients than in healthy controls. In CFS/ME patients, the $BP_{ND}$ values of $^{11}$C-($R$)-PK11195 in the amygdala, thalamus, and midbrain positively correlated with cognitive impairment score, the $BP_{ND}$ values in the cingulate cortex and thalamus positively correlated with pain score, and the $BP_{ND}$ value in the hippocampus positively correlated with depression score. Conclusion: Neuroinflammation is present in widespread brain areas in CFS/ME patients and was associated with the severity of neuropsychologic symptoms. Evaluation of neuroinflammation in CFS/ME patients may be essential for understanding the core pathophysiology and for developing objective diagnostic criteria and effective medical treatments.

Key Words: neuroinflammation; chronic fatigue syndrome (CFS); myalgic encephalomyelitis (ME); $^{11}$C-($R$)-PK11195; positron emission tomography (PET)


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neuroinflammation using a neuroimaging technique such as PET and to investigate the relations between neuroinflammation and symptom severity.

Neuroinflammation is evidenced by activation of microglia or astrocytes, and activated glial cells exhibit an increase in expression of the 18-kDa translocator protein (TSPO). 11C-(R)-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinoline-carboxamide (11C-(R)-PK11195) is a ligand of PET for the TSPO that is expressed in activated microglia or astrocytes and is widely used to assess neuroinflammation in neurologic diseases (7). Recently, it has been reported that although TSPO polymorphisms affect the affinities of second-generation TSPO radioligands, 11C-(R)-PK11195 binds to a different site on TSPO with no apparent difference in affinity (11). Therefore, in the present study, we used 11C-(R)-PK11195 and PET to assess neuroinflammation in patients with CFS/ME. As far as we know, this is the first study to investigate neuroinflammation in CFS/ME patients. Evidence of neuroinflammation in this population would contribute to the clarification of CFS/ME pathophysiology and to the development of objective diagnostic criteria, criteria for evaluation of disease severity, and effective medical treatments.

MATERIALS AND METHODS

Participants

Nine patients who fulfilled the international diagnostic criteria for CFS (12) and ME (13) were recruited from the Fatigue Clinical Center at Osaka City University Hospital, Osaka, Japan. Patients taking medications known to affect autonomic nerve function, including β-blockers, benzodiazepines, corticosteroids, and medications known to affect the central nervous system (e.g., methylphenidate, dexamphetamine, antidepressants, and antipsychotic drugs), were also excluded. Ten age- and sex-matched healthy controls who had no symptoms related to fatigue and no problems in their daily activities were also recruited. Individuals with neuropsychiatric disorders were identified by doctors of general medicine, neurology, and psychiatry at the Osaka City University Hospital and excluded. Demographic and clinical characteristics of the CFS/ME and healthy control groups are shown in Table 1. All participants were right-handed.

The severity of neuropsychologic symptoms was measured using the following questionnaires: a visual analog scale (VAS) for fatigue sensation (14), the Chalder fatigue scale, the Center for Epidemiological Studies depression scale (CES-D), cognitive impairment and pain scores, and duration of disease. The VAS for fatigue sensation ranges from 0 (no fatigue) to 100 (complete exhaustion). The Chalder fatigue scale (15) consists of 11 questions rated on a 4-point Likert scale. The total score ranges from 0 to 33, with higher scores indicating a greater degree of daily fatigue. The CES-D consists of 20 questions rated on a 4-point Likert scale. The total score ranges from 0 to 60, with higher scores indicating a greater degree of depression (16). The cognitive impairment score was assessed using 4 questions on thinking difficulty, inability to concentrate, impairment in memory, and absence of alertness. The pain score was assessed using 4 questions on headache, sore throat, myalgia, and arthralgia. All questions were rated on a 4-point Likert scale, and the total scores range from 0 to 16, with higher scores indicating a greater degree of cognitive impairment and pain (3).

The Ethics Committee of Osaka City University Graduate School of Medicine approved the protocol, and all participants provided written informed consent for participation in the study. This study is registered with the University Hospital Medical Information Network, number UMIN000005469.

Cytokine Assay

Whole-blood samples were collected just before the 11C-(R)-PK11195 PET scan. After collection, blood samples were coagulated at room temperature for more than 30 min and centrifuged at 3,000 rpm for 5 min to obtain sera. Sera were stored at –80°C until measurements. Measurements of serum tumor necrosis factor-α, interferon-γ, interleukin-1β, and interleukin-6 were performed by the Mitsubishi Chemical Medical Corp. The lower detection limit was 0.550 pg/mL for tumor necrosis factor-α, 1.560 pg/mL for interferon-γ, 0.125 pg/mL for interleukin-1β, and 0.300 pg/mL for interleukin-6. Values less than the detection limit were regarded as zero.

MR Imaging

Structural brain images were obtained using a 1.5-T Signa Horizon LX scanner (GE Healthcare) and used to identify the brain regions of interest. Three-dimensional spoiled gradient recalled acquisition was used in the steady-state sequence with the following parameters: repetition time, 17 ms; echo time, 2 ms; flip angle, 40°; and slice thickness, 1.5 mm.

PET Data Acquisition

PET experiments were conducted using an Eminence-B PET scanner (Shimadzu). 11C-(R)-PK11195 was injected during 30 s after the start of the PET scan. The tracer dose was 218.0 ± 31.5 MBq (mean ± SD), with a specific activity of 55.4 ± 22.9 GBq/μmol. Dynamic data were collected over 60 min as 23 temporal frames. Before the dynamic scan, attenuation correction factors were measured with a 10-min transmission scan using an external source of 137Cs. Images were reconstructed with segmented attenuation correction, using Fourier rebinning followed by 2-dimensional filtered back-projection applying a ramp filter cutoff at the Nyquist frequency. The intrinsic spatial resolution was 4 mm in full width at half maximum for the in-slice direction and 6 mm in full width at half maximum for the axial direction. A 3-dimensional gaussian kernel of 6 mm in full width at half maximum was applied for postprocessing. No arterial blood sampling was performed.

Image Data Processing

Structural MR images were coregistered to PET images (summa-
(PMOD Technologies). Each MR image was spatially normalized to the Montréal Neurologic Institute stereotactic brain, and the corresponding PET images were normalized using the same parameters. Thus, the PET and MR images of each participant were anatomically standardized to the Montréal Neurologic Institute template. Because there is no brain region devoid of TSPO (although a cerebellum reference may add stability to quantitative analyses (18)), and because there was no difference between mean time–activity curves of standardized uptake value in CFS/ME patients and healthy controls (Fig. 1C), we generated parametric images of regional \(^{11}\text{C}(-\text{R})\)-PK11195 nondisplaceable binding potential (BP\(_{\text{ND}}\)) using linear graphical analysis according to Logan (19), with the cerebellar cortex as a reference region and corresponding to the linear part of the plot covering the last 40–60 min of measurement (20).

**Image Data Analysis**

All \(^{11}\text{C}(-\text{R})\)-PK11195 BP\(_{\text{ND}}\) images were normalized to the Montréal Neurologic Institute space, smoothed with an isotropic 8-mm gaussian kernel, and analyzed by Statistical Parametric Mapping 5 software (SPM5; Wellcome Department of Cognitive Neurology) using a categoric design. The between-group comparison (CFS/ME patients vs. healthy controls) of \(^{11}\text{C}(-\text{R})\)-PK11195 BP\(_{\text{ND}}\) and correlation analysis of \(^{11}\text{C}(-\text{R})\)-PK11195 BP\(_{\text{ND}}\) values and clinical scores were performed on a voxel-by-voxel basis using \(t\) statistics with the statistical threshold set at a \(P\) value of less than 0.005 at the voxel level and a \(P\) value of less than 0.05 with a correction for multiple comparisons at the cluster level for the entire brain (familywise error). The regions of interest for the cingulate cortex, hippocampus, amygdala, thalamus, midbrain, and pons were defined from the Wake Forest University PickAtlas (21) and applied to the smoothed images.

**Statistical Analyses**

Values are shown as mean ± SD unless stated otherwise. Symptom severity, blood-cytokine data, and regional \(^{11}\text{C}(-\text{R})\)-PK11195 BP\(_{\text{ND}}\) values were compared across the groups using Fisher exact tests. The relation between symptom severity scores and \(^{11}\text{C}(-\text{R})\)-PK11195 BP\(_{\text{ND}}\) values was analyzed using Pearson correlation in the CFS/ME group. All \(P\) values were 2-tailed, and values of less than 0.05 were considered to be statistically significant. Statistical analyses were performed using SPSS 20.0 software (SPSS).

**RESULTS**

**Clinical Scores and Cytokine Data**

The VAS score of fatigue sensation, Chalder fatigue scale score, cognitive impairment score, pain score, and CES-D score were higher in CFS/ME patients than in healthy controls (Table 1). There were some missing cytokine values in the CFS/ME group because of insufficient blood samples (tumor necrosis factor–α missing for 2 participants, and interferon-γ and interleukin-6 missing for 1 participant). In the remaining participants, the concentration of interferon-γ tended to be higher in CFS/ME patients than in healthy controls (1.66 ± 2.77 vs. 0.00 ± 0.00 pg/mL, \(P = 0.0740\)). The concentration of tumor necrosis factor–α (0.80 ± 0.63 vs. 0.45 ± 0.80 pg/mL, \(P = 0.3735\)), interleukin-1β (1.02 ± 0.99 vs. 0.40 ± 0.60 pg/mL, \(P = 0.1129\)), and interleukin-6 (0.60 ± 0.56 vs. 0.75 ± 1.10 pg/mL, \(P = 0.7277\)) was similar between the groups.

\(^{11}\text{C}(-\text{R})\)-PK11195 BP\(_{\text{ND}}\) and Clinical Correlations

Representative maps of \(^{11}\text{C}(-\text{R})\)-PK11195 BP\(_{\text{ND}}\) are presented in Figure 1. \(^{11}\text{C}(-\text{R})\)-PK11195 BP\(_{\text{ND}}\) values for CFS/ME patients (Fig. 1A) were higher than those for healthy controls (Fig. 1B) in widespread brain regions. Region-of-interest analysis revealed that \(^{11}\text{C}(-\text{R})\)-PK11195 BP\(_{\text{ND}}\) values in CFS/ME patients were significantly higher than those in healthy controls in the cingulate, hippocampus, thalamus, midbrain, and pons and tended to be higher in the amygdala (Table 2).

The SPM analysis also revealed significantly higher \(^{11}\text{C}(-\text{R})\)-PK11195 BP\(_{\text{ND}}\) in CFS/ME patients than in healthy controls in the left thalamus, midbrain, and pons (Fig. 2). The Montréal Neurologic Institute coordinates of peak \(^{11}\text{C}(-\text{R})\)-PK11195 BP\(_{\text{ND}}\) (highest \(t\) score on SPM5) in the CFS/ME group corresponded to the intralaminar nucleus of the left thalamus (−6, −22, −4) and midbrain (−4, −30, −14). In the CFS/ME group, the peak value of \(^{11}\text{C}(-\text{R})\)-PK11195 BP\(_{\text{ND}}\) in the left thalamic intralaminar nucleus (−6, −22, −4) was positively correlated with the cognitive impairment score (\(r = 0.86; \ P = 0.0028\)) and tended to be positively correlated with the VAS score for fatigue sensation (\(r = 0.63; \ P = 0.0683\)), and the peak value of \(^{11}\text{C}(-\text{R})\)-PK11195 BP\(_{\text{ND}}\) in the midbrain (−4, −30, −14) was positively correlated with the cognitive impairment score (\(r = 0.72, \ P = 0.0293\)).

In the CFS/ME group, the SPM correlation analysis revealed that \(^{11}\text{C}(-\text{R})\)-PK11195 BP\(_{\text{ND}}\) in the amygdala was positively correlated with cognitive impairment score (Fig. 3A), \(^{11}\text{C}(-\text{R})\)-PK11195 BP\(_{\text{ND}}\) in the hippocampus was positively correlated with...
CES-D score (Fig. 3B), and $^{11}$C-(R)-PK11195 BP$_{ND}$ in the thalamus tended to be correlated positively with pain score (voxel level, $P < 0.005$; cluster level, $P > 0.05$; Fig. 3C). There were no other correlations between $^{11}$C-(R)-PK11195 BP$_{ND}$ in any brain regions and other parameters (age, duration of disease, VAS score of fatigue sensation, score of Chalder fatigue scale, or cytokine concentration).

**DISCUSSION**

The present PET study demonstrated a higher $^{11}$C-(R)-PK11195 BP$_{ND}$ in patients with CFS/ME than in healthy controls in widespread brain regions. $^{11}$C-(R)-PK11195 BP$_{ND}$ in the CFS/ME group was closely related to the severity of neuropsychologic symptoms including fatigue sensation, cognitive impairment, pain, and depression. To our knowledge, this was the first study to provide evidence of neuroinflammation in CFS/ME patients and to demonstrate its possible relation to the pathophysiology of CFS/ME.

Although the mechanisms underlying neuroinflammation in CFS/ME are unclear, one plausible mechanism is overactivity of neurons. To compensate for functional loss associated with CFS/ME, patients have to exert greater effort to perform daily activities, resulting in enhanced neural activation (22). Overactivation of N-methyl-D-aspartate receptors caused by enhanced neural activation results in production of proinflammatory cytokines, reactive oxygen species, and nitrogen species that cause inflammation (23). Another plausible mechanism is the immunologic response to the initial infectious process (24), which can also induce the production of the proinflammatory cytokines, reaction oxygen species, and nitrogen species that cause neuroinflammation (10,25).

Inflammation of the thalamus, particularly of the left intralaminar nucleus, was higher in CFS/ME patients than in healthy controls as shown in Figure 2. Previous studies have discussed the lateralization of $^{11}$C-(R)-PK11195 BP$_{ND}$ that might have resulted from selection bias assessment of predominantly right-handed subjects (26,27). In addition, the level of inflammation in these brain regions was positively associated with cognitive impairment score and fatigue sensation in CFS/ME patients. The intralaminar nucleus of the thalamus and midbrain are involved in the reticular activating system (28), which is considered to play a role in arousal and awareness states. In a previous pharmacologic study of CFS/ME, treatment with clonidine, which controls arousal level through the postsynaptic actions at $\alpha_2$ adrenoreceptors, improved cognitive impairment (29). Inflammation of the thalamus and midbrain may induce cognitive impairment and severe fatigue sensation by perturbing the arousal and awareness states.

Inflammation of the amygdala was also related to the cognitive impairment score in CFS/ME patients. The amygdala receives direct projection from the thalamus, which monitors the sensory information and mediates the facilitation of attention (30). Neuroinflammation in this brain region may reduce cognitive activity through the deterioration of attentional function (31). Although our voxel-based morphometry studies demonstrated volume reduction of bilateral prefrontal cortices in CFS/ME patients (4), we did not focus on neuroinflammation in the prefrontal cortex and did not apply partial-volume correction since the BP$_{ND}$ range of $^{11}$C-(R)-PK11195 in the prefrontal cortex was extremely small ($0.0004$).

In the present study, although CFS/ME patients did not meet diagnostic criteria for major depression or other psychiatric disorders, their depressive scores were higher than those of healthy controls. This finding supports a previous report that depressive symptoms are present in CFS/ME (32). Interestingly, we observed a relation between the severity of depressive symptoms and the level of inflammation in the hippocampus in CFS/ME patients. Neuroinflammation in several brain regions, including

**TABLE 2**

Regional $^{11}$C-(R)-PK11195 BP$_{ND}$ in CFS/ME Patients and Healthy Controls

<table>
<thead>
<tr>
<th>Region</th>
<th>CFS/ME</th>
<th>Healthy control</th>
<th>$P$</th>
<th>Increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midbrain</td>
<td>0.181 ± 0.027</td>
<td>0.123 ± 0.024</td>
<td>0.0001</td>
<td>47</td>
</tr>
<tr>
<td>Pons</td>
<td>0.155 ± 0.030</td>
<td>0.107 ± 0.028</td>
<td>0.0021</td>
<td>45</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.097 ± 0.021</td>
<td>0.058 ± 0.023</td>
<td>0.0013</td>
<td>66</td>
</tr>
<tr>
<td>Cingulate</td>
<td>0.010 ± 0.008</td>
<td>0.003 ± 0.003</td>
<td>0.0535</td>
<td>199</td>
</tr>
<tr>
<td>Amygdala</td>
<td>0.057 ± 0.031</td>
<td>0.031 ± 0.025</td>
<td>0.0586</td>
<td>85</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.053 ± 0.023</td>
<td>0.029 ± 0.017</td>
<td>0.0212</td>
<td>81</td>
</tr>
</tbody>
</table>

Data are mean ± SD. Student $t$ tests or Fisher exact test was conducted.
Remains unclear. It is difficult to accurately measure the levels of neuroinflammation and peripheral proinflammatory cytokines with deterioration, in CFS/ME patients. Also, the relation between patients have already started this type of combined PET study on the same their interactions associated with pain in CFS/ME patients. We would clarify inflammatory and serotonergic mechanisms and their interactions associated with pain in CFS/ME patients.

Further studies are needed for confirmation of this finding of the existence of neuroinflammation, and its correlation in some way with deterioration, in CFS/ME patients. Also, the relation between neuroinflammation and peripheral proinflammatory cytokines remains unclear. It is difficult to accurately measure the levels of serum cytokines because of their short half-lives and the degradation of molecules that may occur during the storage of blood samples (36). This suggests that neuroinflammation should be assessed using PET rather than by measuring peripheral proinflammatory cytokines. However, analysis for the BPND of 11C-(R)-PK11195 had several limitations. Although we showed no change in the identical standardized uptake value curves of the reference region (cerebellum) between groups (Fig. 1C), these curves are not proof of no changes in the reference region, as there could be between-group input function differences; arterial input functions might have confirmed this issue more authoritatively. The BPND values of 11C-(R)-PK11195 were low. This result is highly dependent on there being no differences in the reference region. 11C-(R)-PK11195 is known to offer a poorer signal-to-noise ratio than the second-generation radioligands for TSPO such as 11C-PBR28 (37). Therefore, we are currently performing the next-phase international collaboration study using 11C-PBR28 with arterial input function to evaluate neuroinflammation of CFS/ME patients in relation to other neurotransmitter dysfunctions. Furthermore, we hope that more specific radioligands for TSPO or glial cells, such as 11C-(S)-ketoprofen-methyl ester, which is a good radiotracer for cyclooxygenase-1 imaging in brain microglia activation (38), will provide more information on neuroinflammation in CFS patients.

CONCLUSION
Our results provide evidence of neuroinflammation in CFS/ME patients, as well as evidence of the possible contribution of neuroinflammation to the pathophysiology of CFS/ME. Furthermore, our results demonstrate the usefulness of PET imaging for the development of objective diagnostic criteria, evaluation of disease severity, and effective medical treatment strategies using antiinflammatory agents in CFS/ME.

DISCLOSURE
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