[11C]-(R)-PK11195 positron emission tomodraphy in patients with complex regional pain syndrome

A pilot study

So Yeon Jeon, MD, a Seongho Seo, PhD, b,c, Jae Sung Lee, PhD, d,e, Do-Hyeong Lee, MD, a Ye-Ha Jung, PhD, a Man-Kyu Song, MD, a Kyung-Jun Lee, MD, a Yong Chul Kim, MD, PhD, f Hyeon Woo Kwon, MD, g, Hyung-Jun Im, MD, g, Dong Soo Lee, MD, PhDb,g, Gi Jeong Cheon, MD, PhDb,d, Do-Hyung Kang, MD, PhDb,e.

Abstract

Complex regional pain syndrome (CRPS) is characterized by severe and chronic pain, but the pathophysiology of this disease are not clearly understood. The primary aim of our case–control study was to explore neuroinflammation in patients with CRPS using positron emission tomodraphy (PET), with an 18-kDa translocator protein specific radioligand [11C]-(R)-PK11195. [11C]-(R)-PK11195 PET scans were acquired for 11 patients with CRPS (30–55 years) and 12 control subjects (30–52 years). Parametric image of distribution volume ratio (DVR) for each participant was generated by applying a relative equilibrium-based graphical analysis. The DVR of [11C]-(R)-PK11195 in the caudate nucleus (t(23)=-3.209, P=0.004), putamen (t(23)=-2.492, P=0.022), nucleus accumbens (t(23)=-2.218, P=0.040), and thalamus (t(23)=-2.395, P=0.026) were significantly higher in CRPS patients than in healthy controls. Those of globus pallidus (t(23)=-2.045, P=0.054) tended to be higher in CRPS patients than in healthy controls. In patients with CRPS, there was a positive correlation between the DVR of [11C]-(R)-PK11195 in the caudate nucleus and the pain score, the visual analog scale (r=0.661, P=0.026, R$^2=0.408$) and affective subscales of McGill Pain Questionnaire (r=0.604, P=0.049, R$^2=0.364$). We demonstrated that neuroinflammation of CRPS patients in basal ganglia. Our results suggest that microglial pathology can be an important pathophysiology of CRPS. Association between the level of caudate nucleus and pain severity indicated that neuroinflammation in this region might play a key role. These results may be essential for developing effective medical treatments.

Abbreviations: BAI = Beck Anxiety Index, BG = basal ganglia, CRPS = complex regional pain syndrome, DVR = distribution volume ratio, MPQ_A = McGill pain Questionnaire Affective, MRI = magnetic resonance imaging, PET = positron emission tomodraphy, ROI = region of interest, SF-MPQ = McGill pain questionnaire Short-form, TSPO = translocator protein, VAS = visual analog scale.

Keywords: central sensitization, complex regional pain syndrome, microglia, neuroinflammation, PK11195-PET

1. Introduction

Complex regional pain syndrome (CRPS) is a debilitating pain condition characterized by chronic severe pain, allodynia, hyperalgesia, motor, and autonomic signs and symptoms, as well as changes in emotional and cognitive function. Clinically, CRPS patients are profoundly affected with decreased ability to participate in normal activities of daily living.

Although the pathophysiology of CRPS has not been clearly defined, CRPS is thought to involve peripheral and central
sensitization of neuronal function. Following nerve injury, a cascade of events is initiated that includes peripheral inflammation and potentially increased infiltration of the central nervous system (CNS). The CNS undergoes functional and structural changes in people with persistent pain. These changes are thought to be particularly important in patients with CRPS and can lead to central sensitization over the long term. In CRPS patients, features of central sensitization appear to involve a complex set of neuroinflammatory cellular responses, known as “giall cell activation.” Normally, glial activation is an adaptive defensive mechanism contributes to handling of acute stress, limiting tissue damage, and restoring homeostasis. However, malfunctioning glial activation can have deleterious effects and become the primary pathogenic condition. Activated microglia are an important source of inflammatory mediators, thus may play fundamental roles in neuropathic pain.

In a previous study, a cohort of chronic pain patients exhibited persistent microglial activation in their contralateral thalamus by using [11C]-[(R)-(R)-PK11195 positron emission tomography (PET). Conversely, the injection of drugs inhibiting glial activation can inhibit, delay, or reverse pain. Therefore, ongoing CNS inflammation mediated by glial activation is a possible contributing factor to the manifestation and maintenance of the psychophysical/behavioral responses observed in CRPS patients.

Evidence of neuroinflammation is supported by the existence of activated microglia or astrocytes. Activated glial cells demonstrate an increased expression of the 18-kDa translocator protein (TSPO). [11C]-[(R)-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isouquinoline-carboxamide PK11195 ([11C]-[(R)-(R)-PK11195] is a TSPO-specific radioligand. The normal brain shows relatively low tissue-specific binding of [11C]-[(R)-(R)-PK11195, as the microglia are in a resting state. [11C]-[(R)-(R)-PK11195 binding reflects the distribution of microglia activated by brain injury in conditions such as multiple sclerosis and chronic back pain. [11C]-[(R)-(R)-PK11195 PET is widely used to assess neuroinflammation in neurological diseases (e.g., Alzheimer disease and idiopathic Parkinson disease). Although affinities of second-generation TSPO radioligands are known to be varied according to TSPO polymorphism, [11C]-[(R)-(R)-PK11195 binds to another site on TSPO which is not involved in PK11195 binding properties, resulting in no apparent difference in affinity. Banati et al. reported an imaging study using [11C]-[(R)-(R)-PK11195 as a biomarker to visualize neuroinflammation in the CNS in vivo. This study indicated that peripheral nerve injury resulted in microglial activation in CNS at the first-order synapses of the injured neurons, and some of this activation propagated to the second-order synapses located in the thalamus. We reviewed studies pertaining to chronic pain, neuroinflammation, and central sensitization to establish a priori hypotheses. In central sensitization, neuroinflammation has been observed in the thalamus. In CRPS, putative roles of the basal ganglia (BG) include motor dysfunction as well as alterations in mood, including reward dysfunction. Furthermore, CRPS patients, especially in type I, exhibit changes in somatosensory systems processing noxious, tactile, and thermal information. The somatosensory system involving pain perception includes the thalamus, the primary somatosensory cortex, and the insula. Patients with chronic pain often experience elevated activation of brain regions involved in cognitive and/or emotional pain processing. Therefore, we hypothesized that neuroinflammation would increase in the thalamus, BG, primary somatosensory cortex, insula, and regions involved in pain processing.
2.2. Positron emission tomography/magnetic resonance image acquisition

Each participant underwent a 60-minute dynamic \(^{11}C\)-(R)-PK11195 PET scan using a Siemens Biograph mMR (Siemens medical solution, Knoxville, TN, USA) PET/MRI scanner (PET spatial resolution: 4.4 mm at 1 cm and 5.2-mm full width half maximum [FWHM] at 10-cm offset from the center of the transverse field-of-view). \(^{11}C\)-(R)-PK11195 (503 ± 74 MBq) was administered with an intravenous bolus injection, and the PET data were acquired in a 3-dimensional (3D) list mode without arterial blood sampling. A total of 27 dynamic PET frames (12 frames × 5 seconds, 5 × 60, 3 × 180, 5 × 300, and 2 × 600) were reconstructed using filtered back projection followed by 4-mm FWHM Gaussian postfiltering, with routine corrections for physical effects such as radioactive decay and attenuation (using an ultrashort-echo-time MR-based attenuation map).

2.3. Quantification of binding

A parametric image of the DVR for each participant was generated by applying the relative equilibrium-based graphical analysis based on a reference region to the reconstructed dynamic images.\(^{10}\) The time interval for the linearity of the graphical analysis was 30 to 60 minutes for all voxels, and the cerebellum was used as a reference region. The reference input function was obtained by delineating the region of interest (ROI) of the cerebellum on an individual T1-weighted MRI (256 × 256 matrix; 208 sagittal slices; voxel size: 0.98 × 0.98 × 1.00 mm\(^3\)). The ROI was then placed on the reconstructed dynamic PET images. We did not apply coregistration between PET and MRI because the T1 MRI was simultaneously acquired with the PET data using a 3D Turbo FLASH sequence (repetition time: 1670 ms; echo time: 1.89 ms; flip angle: 9\(^\circ\)). For delineating the ROI, we used the FMRIB Integrated Registration and Segmentation Tool (FIRST, FSL v4.0, Oxford University; Oxford, UK, http://www.fmrib.ox.ac.uk/fsl). The regional average DVR of ROI were extracted from the spatially normalized unsmoothed DVR images using population-based probability maps,\(^{11,12}\) and the mean value for both hemispheres of DVR was used in analyses.

2.4. Synthesis of \(^{11}C\)-(R)-PK11195 PET

\(^{11}C\)-(R)-PK11195 was synthesized using the previously reported method with a few modifications.\(^{13}\) The precursor and the cold-standard of \(^{11}C\)-(R)-PK11195 were purchased from ABX (Radeberg, Germany); the other chemicals were obtained from Sigma-Aldrich Korea (Kyunggi-do, Korea). Briefly, (R)-N-desmethyl-PK11195 (the precursor of \(^{11}C\)-(R)-PK11195, 1mg, 2.8\(\mu\)mol) and sodium hydride (60% in mineral oil, 7 mg, 486\(\mu\)mol) were dissolved in 500 \(\mu\)L of dimethyl sulfoxide in a reaction vial. \(^{11}C\)CH\(_3\)I was bubbled into the reaction vial for 10 minutes, and the reaction mixture was allowed to proceed for an additional 5 minutes. The reaction mixture was purified by preparative high-performance liquid chromatography (HPLC) (Xterra C18 column, 10 × 250 mm\(^2\), 10\(\mu\)m, Waters Corp.; Milford, MA) with the eluent (water: ethanol = 55:45, 5 mL/min). The final product solution was constituted with 10 mL of a saline solution. The purity of the final product was checked by analytical HPLC (YMC-Triart C18 column, 4.6 × 100 mm\(^2\), 3 \(\mu\)m, YMC Co., Ltd.; Kyoto, Japan) with the eluent (water:MeCN = 40:60, 1 mL/min). The radioactivity of \(^{11}C\)-(R)-PK11195 was 5.7 ± 1.8 GBq (156.7 ± 51.3 mCi) [n = 10]; the specific activity was 2152.5 ± 93.7 GBq/\(\mu\)mol (5.8 ± 2.5 Ci/mol [n = 10]). The total synthesis time, including formation and purification, was less than 40 minutes from the end of bombardment.

2.5. Statistical analysis

Statistical analyses were performed using SPSS Statistics 21.0 (Chicago, IL). For all the variables, the normality of the distribution was tested with the Kolmogorov–Smirnov test. If normality was satisfied, differences between 2 groups were tested using the Student 2-tailed t test, and bivariate correlation was tested using Pearson correlation coefficient. If normality was not satisfied, Mann–Whitney U test and the Spearman rho correlation coefficient were used. Regional average DVR of \(^{11}C\)-(R)-PK11195 were assessed using a Student 2-tailed t test. A Pearson correlation analysis was used to evaluated the association between the DVR of \(^{11}C\)-(R)-PK11195 and the neuropsychiatric assessment scale and subdomain of SF-MPQ. The P value deemed to indicate significance was set at 0.05.

3. Results

3.1. Study participants

Fifteen subjects were enrolled in each group, but 11 participants with CRPS and 12 healthy control subjects completed all study procedures. Three CRPS patients and 2 of the healthy control subjects withdrew their consent. Synthesis of the \(^{11}C\)-(R)-PK11195 precursor failed once in each group. Demographic and clinical characteristics of the subjects are presented in Table 1. Age, the ratio of sex, education, and hs-CRP levels were not

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant’s demographic and clinical characteristics.</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Age, y</td>
</tr>
<tr>
<td>Sex (n)</td>
</tr>
<tr>
<td>Education, y</td>
</tr>
<tr>
<td>hs-CRP, mg/dL</td>
</tr>
<tr>
<td>Duration of illness, y</td>
</tr>
<tr>
<td>BDI</td>
</tr>
<tr>
<td>BAI</td>
</tr>
</tbody>
</table>

Data are shown as mean ± standard deviation (range).\[^{14}\] BAI = Beck Anxiety Index, BDI = Beck depression index, CRPS = complex regional pain syndrome, F = female, hs-CRP = high-sensitivity C-reactive protein, M = male.\[^{15}\] P value derived using 2-sample independent t test (\(*\) t-test for sex).

[^14]: www.md-journal.com
significantly different between 2 groups. CRPS patients had much higher BDI and BAI scores compared with the healthy control subjects.

3.2. Comparison of $[^{11}C]-R$-PK11195 DVR between the CRPS patients and healthy control subjects

Consistent with our a priori hypotheses, participants with CRPS had a significantly higher $[^{11}C]-R$-PK11195 DVR than the healthy control subjects in the caudate nucleus ($P=0.004$), putamen ($P=0.021$), nucleus accumbens ($P=0.040$), and thalamus ($P=0.026$). Although it was not statistically significant, those of globus pallidus ($P=0.054$) and postcentral gyrus ($P=0.059$) tended to be higher in CRPS patients than in healthy controls (Figs. 1 and 2, Table 2). Contrary to our expectations, there were no significant DVR differences between 2 groups in amygdala ($P=0.998$), cingulate cortex ($P=0.292$), insula ($P=0.232$), and subthalamic nucleus ($P=0.321$).

3.3. Correlation between $[^{11}C]-R$-PK11195 DVR and pain severity in CRPS patients

Our study found significant correlation between pain severity and $[^{11}C]-R$-PK11195 DVR of caudate nucleus among CRPS patients. There is a positive correlation between the $[^{11}C]-R$-PK11195 DVR and the VAS ($r=0.661$, $P=0.026$, $R^2=0.408$) (Fig. 3A), as well as the MPQ_A scores ($P=0.604$, $P=0.049$, $R^2=0.364$) (Fig. 3B). There were no significant correlations between $[^{11}C]-R$-PK11195 DVR of other regions and pain severity. Either, no association was found between $[^{11}C]-R$-PK11195 DVR and hs-CRP, illness duration or other neuropsychiatric assessment scores was observed.

4. Discussion

Neuroinflammation, as indexed by $[^{11}C]-R$-PK11195 DVR, was significantly higher in participants with CRPS than in healthy control subjects. In support of our a priori hypothesis, significant increases in the $[^{11}C]-R$-PK11195 DVR were observed in the caudate nucleus, putamen, nucleus accumbens, and thalamus. In addition, correlation analysis revealed that $[^{11}C]-R$-PK11195 DVR in the caudate nucleus was associated with higher VAS and MPQ_A scores. Our findings support a role for microglial activation in central sensitization, which could be an underlying contributor to the manifestation and maintenance of psycho-physical, behavioral responses, and chronic pain observed in CRPS patients.

Our results are consistent with previously published neurogenic inflammation studies that indicated increased levels of proinflammatory cytokines using methodologically diverse animal and human studies. Although the mechanisms underlying neuroinflammation in CRPS are unclear, 1 plausible explanation is neuronal overactivity. In particular, microglia showed increased activity in pain hypersensitivity following peripheral

### Table 2

<table>
<thead>
<tr>
<th>Region</th>
<th>Healthy controls</th>
<th>CRPS</th>
<th>$P$ (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdala</td>
<td>$0.97\pm0.05$</td>
<td>$0.97\pm0.08$</td>
<td>$0.098^*$</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>$0.76\pm0.08$</td>
<td>$0.86\pm0.06$</td>
<td>$0.004^*$</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>$0.99\pm0.05$</td>
<td>$1.04\pm0.06$</td>
<td>$0.054^*$</td>
</tr>
<tr>
<td>Putamen</td>
<td>$0.98\pm0.02$</td>
<td>$1.02\pm0.05$</td>
<td>$0.021^*$</td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td>$0.96\pm0.07$</td>
<td>$1.03\pm0.08$</td>
<td>$0.040^*$</td>
</tr>
<tr>
<td>Thalamus</td>
<td>$1.04\pm0.07$</td>
<td>$1.10\pm0.06$</td>
<td>$0.026^*$</td>
</tr>
<tr>
<td>Subthalamic nucleus</td>
<td>$1.15\pm0.08$</td>
<td>$1.19\pm0.08$</td>
<td>$0.321^*$</td>
</tr>
<tr>
<td>Cingulate cortex</td>
<td>$0.86\pm0.04$</td>
<td>$0.90\pm0.05$</td>
<td>$0.292^*$</td>
</tr>
<tr>
<td>Postcentral gyrus</td>
<td>$0.82\pm0.04$</td>
<td>$0.86\pm0.06$</td>
<td>$0.059^*$</td>
</tr>
<tr>
<td>Precentral gyrus</td>
<td>$0.84\pm0.04$</td>
<td>$0.87\pm0.06$</td>
<td>$0.080^*$</td>
</tr>
<tr>
<td>Insula</td>
<td>$0.92\pm0.04$</td>
<td>$0.95\pm0.06$</td>
<td>$0.232^*$</td>
</tr>
</tbody>
</table>

Data are mean±SD. Independent $t$ test was conducted. $^*$Significantly different between 2 groups ($P<0.05$).
support of these findings, a study in another chronic pain disease, fibromyalgia, demonstrated a decreased regional cerebral blood flow in the caudate nucleus using brain single-photon emission computed tomography.\textsuperscript{43} Previous studies have suggested 2 putative roles for the caudate nucleus in pain: to reduce the pain in affected components elicited by noxious stimuli,\textsuperscript{42} and pain avoidance behavior.\textsuperscript{44} Decreased activation of the caudate nucleus was previously observed in response to mechanical, cold, and heat allodynia in chronic pain conditions.\textsuperscript{45} Similarly, functional imaging in a group of pediatric patients with CRPS also showed a decrease in activation in the caudate nucleus.\textsuperscript{46}

Taken together, these data appear to support a neuroinflammation followed by decreased activation of the caudate nucleus in response to pain produced through multiple painful stimuli and is related to the affective component of pain.

BG has been indicated as an important region which take a part in pain processing, including affective, cognitive dimension of pain, and pain modulation.\textsuperscript{42} Studies in patients with lesions of the BG have implied the potential role of BG in pain and analgesia. Patients with infarction of the lenticular nucleus sometimes show sensory deficits.\textsuperscript{47} Deep brain stimulation of the globus pallidus has been reported to alleviate pain symptoms by approximately 70%, and this improvement may persist for a long duration.\textsuperscript{48} Multisensory integration in the BG serves as an important component for the modulation of cortical information to increase data-processing speed.\textsuperscript{49} In acute/chronic pain response, data-processing is fastened in same manner, by the sensory, motor, autonomic, cognitive, and emotional integration.\textsuperscript{50} This mechanism could explain the various symptoms experienced by patients with CRPS. Our results also showed a significant correlation between \([11\text{C}]\)-\((R)\)-PK11195 DVR in the caudate nucleus and pain severity (Fig. 3). Thus, these findings provide an initial understanding of the potential role of the BG in chronic pain disorders.

The thalamus is a key region involved in pain processing; increased neuroinflammation in this region could explain central sensitization of chronic pain in CRPS. Consistent with our results, neuroinflammation of the thalamus has been reported in chronic fatigue syndrome/myalgic encephalomyelitis patients using \([11\text{C}]\)-\((R)\)-PK11195 PET\textsuperscript{51} and in chronic back pain patients using \([11\text{C}]\)-PBR28 PET.\textsuperscript{52}

The paramedian and anterolateral parts of the thalamus are known to be involved with central pain processing. The striatum receives excitatory signals from the thalamus, while the centromedian and parafascicular thalamic nuclei send connections to the putamen and caudate via thalamostriatal projection.\textsuperscript{50} Although not yet observed in the human brains of chronic pain patients, preclinical studies have evaluated various receptor associated with inflammation (Interleukin (IL)-6, IL-1β, IL-18, and N-methyl-D-aspartate receptors) in the thalamus of mice. The study shows altered reactivity to pain, stress, and anxiety-related behaviors.\textsuperscript{50}

Pain engages emotion/reward/motivational circuitry (e.g., the nucleus accumbens). Induction of acute emotional or physical pain in patients with chronic pain conditions, through exposure to reminders of the deceased or thermal pain, robustly engages the key reward and reinforcement structure.\textsuperscript{51} In the study presented here, we did not observe correlations between DVR uptake in the NAc and pain severity, depressed mood, and/or anxiety symptoms; however, the NAc showed significantly higher uptake of DVR in CRPS patients than in healthy controls. These findings could suggest that persistent neuroinflammation in this region results in a chronic pain state and induces an altered

![Figure 3. Relationships between \([11\text{C}]\)-\((R)\)-PK11195 distribution volume ratio (DVR) and pain severity scores in CRPS patients. Positive associations were seen between \([11\text{C}]\)-\((R)\)-PK11195 DVR in caudate nucleus and VAS (A).](image)

A

B

Figure 3. Relationships between \([11\text{C}]\)-\((R)\)-PK11195 distribution volume ratio (DVR) and pain severity scores in CRPS patients. Positive associations were seen between \([11\text{C}]\)-\((R)\)-PK11195 DVR in caudate nucleus and VAS (A). Pearson coefficient value (r) and P value are shown for each relation. VAS = visual analog scale, MPQ_A = McGill Pain Questionnaire_Affective.
hedonic state, disorder of pleasure evaluation, and dysfunction of reward processing. The results of the present study indicated increased DVR in the postcentral gyrus, a primary somatosensory cortex, in CRPS patients compared with the healthy control group. This increased uptake may be related to cortical reorganization in CRPS. Studies using structural MRI have reported significant cortical shrinkage of the somatosensory cortex in patients with chronic back pain and CRPS patients.

Several limitations of this study should be discussed. Our work only examined a small number of CRPS type I patients. Thus, future studies with larger sample size will be required to generalize this finding to CRPS patients as a whole. The CRPS patients included in this study were taking analgesic agents, including opioids, during the study period, and we were therefore unable to exclude this analgesic effect in the results. However, we did examine subjects who were not taking benzodiazepine drugs, which directly affects TSPO-binding affinity, for at least 2 weeks before the [11C]-PK11195 PET examination to minimize medication effect.

In conclusion, this is the first in vivo investigation of neuroinflammation using [11C]-PK11195 PET imaging in CRPS patients. The increased DVR was detectable in patients many years after injury, indicating long-term reorganization of these brain regions. This glial activation may reflect continually changed afferent activity, even beyond the first-order projection area of the injured neurons. These results provide evidence of neuroinflammation, potentially a key mechanism underlying the central sensitization in CRPS and highlight a fascinating area for future research.

Acknowledgments

The authors thank all the participants in this study, especially from the CRPS support organization, Korea.

References


Elman I, Zubieta J-K, Borsook D. The missing p in psychiatric training: why it is important to teach pain to psychiatrists. Arch Gen Psychiatry 2011;68:12–20.