Reduced Cerebral Perfusion of Putamen and Insular Cortex in Patients with Idiopathic Restless Leg Syndrome

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Objectives: To evaluate the cerebral perfusion pattern of idiopathic restless leg syndrome (RLS). Methods: We performed Tc-ethyl cysteinate dimer brain single-photon emission computed tomography (SPECT) in 36 drug-naive patients with RLS patients and 30 age and gender matched normal controls during wakefulness. Their SPECT images underwent statistical parametric mapping analysis. Results: Mean age of patients and normal controls was 48.3 years and 80% of them were women. Most patients reported the sleep onset and maintenance insomnia due to RLS symptoms. Average duration of RLS was 10.5 years. Mean score of international RLS was 26.6, suggesting moderate to severe severity. They did not report daytime sleepiness (mean Epworth sleepiness scale, 7.0). All subjects underwent polysomnography, showing no definite obstructive sleep apnea syndrome. Seventeen of them (56.6%) had periodic leg movement during sleep (PLMS). Sleep quality was more deteriorated in patients than that in normal controls (increased sleep latency and arousal index, which resulted in reduced sleep efficiency). In RLS patients, cerebral blood flow was decreased in the right putamen and insular cortex compared to normal controls (uncorrected p<0.005). There was no brain region showing increased cerebral perfusion in patients. Regional cerebral perfusion was not correlated to any sleep parameter including PLMS index or movement arousal index. Conclusions: Reduced cerebral perfusion in putamen and insular cortex in RLS suggested functional abnormalities in motor circuit and sensori-motor modulation, which may be related to pathophysiology of idiopathic RLS.

Key Words: RLS, Cerebral perfusion, SPECT, Statistical parametric mapping.

Introduction

Restless legs syndrome (RLS) is characterized by a disagreeable sensation in the legs coupled with an urgent desire to move the limbs. It mainly occurs at rest in the evening and at night, thus impairing sleep initiation and maintenance. The most characteristic clinical feature is the occurrence of circadian disabling sensory symptoms at rest, responsive in most patients to dopaminergic drugs. Most attempts to document any dopamine pathology in RLS to date have been unconvincing. Brain imaging studies have failed to provide a consistent pattern indicating a dopamine deficit.

An autopsy study found that the dopaminergic profile in the putamen and substantia nigra of individuals with RLS have a number of significant differences from controls. Very recently 11C-methylphenidate and positron emission tomography (PET) study assessed real-time dopamine transporter binding potentials in striatum of RLS patients and showed significantly lower binding in the striatum in RLS patients compared to controls.

Another important feature is that RLS remains one of the most intriguing and commonest chronic sensory-motor disorders. Although normal lower extremity (posterior tibial nerve stimulation) and upper extremity (median nerve stimulation) somatosensory evoked responses have been reported in RLS patients. But these findings do not provide any evidence for a primaryafferent sensory disturbance and indirectly support an abnormal sensory processing at a peripheral or central level associated with an abnormal sensory-motor integration.

To our knowledge, there was no report to investigate the cerebral perfusion in patients with primary RLS. Here we examined drug-naive patients with RLS, and by means of Tc-ethyl cysteinate dimer (Tc-ECD) brain single-photon emission computed tomography (SPECT) to evaluate a possible association of cerebral perfusion abnormalities and RLS sym-
Patients and Methods

Patients

Thirty patients with idiopathic RLS and 30 age-and sex matched normal controls were recruited. Inclusion criteria of patients were as follows: 1) older than 18 but younger than 55 years old, 2) an apnea-hypopnea index less than 5 per hour, and 3) serum ferritin concentration above 70 µg/mL. Normal controls were recruited using an advertisement in a local community. Each candidate had a detailed clinical interview, sleep questionnaire, and overnight PSG. If a healthy volunteer candidate has the evidence of obstructive sleep apnea syndrome (apnea-hypopnea index greater than 5 if airflow measured by thermistor), or evidence for other sleep disorders, he/she was excluded. Exclusion criteria for patients and normal controls were those with 1) mean daily sleep time <6 hours, 2) abnormal sleep-wake rhythm, 3) other sleep disorders (OSA, parasomnia, hypersomnia, etc), 4) hypertension, diabetes, heart, and respiratory diseases, 5) history of cerebrovascular disease, 6) other neurological (neurodegenerative diseases, epilepsy, head injury) or psychiatric diseases (psychosis, current depression), 7) alcohol or illicit drug abuse or current intake of psychoactive medications, and 8) a structural lesion on brain magnetic resonance imaging (MRI).

Overnight polysomnography

The day before sleep studies, patients were asked not to drink alcohol or caffeinated beverages. Sleep studies were recorded using a Somnologica (Embla, Denver, CO, USA). Overnight polysomnography was performed using a four-channel electroencephalogram (EEG, C3/A2; C4/A1; O1/A2; O2/A1), a four-channel electrooculogram, an electromyogram (of submental, intercostal, and anterior tibialis muscles), and an electrocardiogram with surface electrodes. A thermistor (for monitoring nasal airflow), a nasal air pressure monitor, an oximeter (for measuring oxygen saturation), piezoelectric bands (for determining thoracic and abdominal wall motion), and a body position sensor were also attached to patients. Patients were recorded on videotape using an infrared video camera and were continuously observed by a polysomnography technician. Patients went to bed at 11:00 PM and were awakened at 7:00 AM. Sleep architecture was scored in 30-sec epochs, and sleep staging was interpreted according to the standard criteria of Rechtschaffen and Kales. Apneas and hypopneas were defined by criteria. An obstructive apnea was defined as a reduction in airflow greater than 90% lasting at least 10-sec during which there was evidence of a persistent respiratory effort. A central apnea was defined as a reduction in airflow of more than 90% lasting at least 10-sec during which there was no evidence of respiratory effort. A hypopnea was defined as a reduction in airflow by a reduction of airflow by 30% for more than 10-sec, accompanied by EEG arousal and/or a 4% or greater oxygen desaturation. According to the American Sleep Disorders Association Task Force criteria, arousals are classified as breathing-related arousals (occurring within 3-sec following apnea, hypopnea, or snoring) and other type of arousals (spontaneous arousal, movement arousal index).

99mTc-ECD brain SPECT

99mTc-ethyl cysteinate dimer was injected intravenously for the SPECT studies. A brain SPECT scan was performed within 30 to 60 min of radiotracer injection (25 mCi) using a 3-headed Triad XLT system equipped with low-energy and high-resolution collimators (Trixion Research Laboratory, Twinsburg, OH, USA). The transaxial system resolution of this camera was 6.9 mm full width at half maximum. Images were reconstructed by filtered back-projection using a Butterworth filter. Attenuation correction was performed using Chang’s method (attenuation coefficient=0.12 cm-1). The SPECT voxel dimension was 3.56×3.56×3.56 mm (x, y, z). All participants were asked to refrain from drinking caffeinated beverages but were allowed to drink water from 07:00 until the end of the SPECT study. Patients were instructed not to fall asleep after ECD injection. Wakefulness after an ECD injection was monitored using a 4-channel electroencephalogram, a 2-channel electrooculogram, and 1-channel electromyogram. Informed consent was obtained from all study subjects after the study protocol, the SPECT procedure, and the potential hazards of radioisotope injection had been explained. The Institutional Review Board at Samsung Medical Center authorized the informed consent form used and the study protocol, which included the administration of a radioactive substance and SPECT scanning.

SPM analysis of SPECT studies

Single-photon emission computed tomography images of the PLMD patients and normal controls were manipulated using MATLAB 7.1 (The MathWorks, Natick, MA, USA) incorporated into SPM 2 software (Wellcome Department of Cognitive Neurology, Institute of Neurology, University of London, UK). Raw SPECT images (interfile 3.0 formats) were converted to 16 bits Analyze format. All of the SPECT images of the healthy volunteers and syncope patients were spatially normalized to the standard SPECT template with the default options. The accuracy of the spatial normalization was checked using a cross-registration function. Spatially normalized images were then smoothed by convolution using an isotopic...
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Gaussian kernel with a 14-mm full width at half maximum to increase the signal to noise ratio. The count of each voxel was normalized to the mean intensity of the white matter to remove differences in the global cerebral blood flow (CBF) between individuals. The results were superimposed on the 2-D planes of the averaged MRI template of normal subjects which was normalized to Montreal Neurological Institute space.

Table 1. The results of overnight polysomnography in patients with restless leg syndrome (RLS) and normal controls

<table>
<thead>
<tr>
<th></th>
<th>RLS patients</th>
<th>Normal controls</th>
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<tbody>
<tr>
<td>Total sleep time (min)</td>
<td>355.1 (40.1)</td>
<td>385.5 (41.1)</td>
<td>0.498</td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>75.3 (11.5)</td>
<td>92.5 (3.5)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Sleep latency (min)</td>
<td>45.1 (10.5)</td>
<td>7.8 (5.0)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>REM sleep latency (min)</td>
<td>130.5 (38.5)</td>
<td>86.0 (15.8)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Apnea-hypopnea index (per hr)</td>
<td>5.9 (1.5)</td>
<td>2.3 (2.1)</td>
<td>0.057</td>
</tr>
<tr>
<td>Arousal index (per hr)</td>
<td>20.5 (5.5)</td>
<td>5.2 (3.1)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Total PLMS index (per hr)</td>
<td>25.3 (10.2)</td>
<td>2.8 (5.5)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Movement arousal index (per hr)</td>
<td>4.3 (1.9)</td>
<td>0.3 (1.4)</td>
<td>0.002*</td>
</tr>
<tr>
<td>NREM 1%</td>
<td>19.6 (4.3)</td>
<td>9.5 (4.7)</td>
<td>0.015*</td>
</tr>
<tr>
<td>NREM 2%</td>
<td>63.2 (10.0)</td>
<td>48.5 (12.5)</td>
<td>0.032*</td>
</tr>
<tr>
<td>NREM 3%</td>
<td>3.5 (5.8)</td>
<td>9.7 (4.5)</td>
<td>0.020*</td>
</tr>
<tr>
<td>REM %</td>
<td>12.5 (4.2)</td>
<td>23.8 (7.3)</td>
<td>0.024*</td>
</tr>
</tbody>
</table>

Mean values (standard deviation), *independent t-test, p<0.05. PLMS: periodic limb movement during sleep, REM: rapid eye movement, NREM: non-rapid eye movement

Statistics
A one-way ANCOVA with covariates of age and gender was used for the regional CBF (rCBF) difference between the two groups. The voxel clusters were corrected with an extent threshold of $k_1 > 100$ voxels.

Results

Clinical and polysomnographic characteristics
In RLS patients, most were middle-aged (mean age 48.3 years, 24 female) and normal body weighted (mean body mass index=24.5 kg/m$^2$). The mean onset age of RLS insomnia related to RLS symptoms was 41.5 years (range 29-50), and the mean duration of presumed RLS was 10.5 years (range 4-29). Mean score of international RLS was 26.6, suggesting moderate to severe severity. They did not report daytime sleepiness (mean Epworth sleepiness scale, 7.0). Compared to normal controls, RLS patients showed poorer sleep quality. Detailed PSG data of subjects were summarized and compared in Table 1.

Wakefulness monitoring during SPECT studies
Electroencephalograms, electrooculograms, and electromyograms were monitored during SPECT studies to measure the duration of wakefulness after an ECD injection in all patients. Mean sleep latency after an ECD injection was 12.5±1.3 minutes, (range 9.5-20). Thus, we confirmed that all subjects underwent brain SPECT during the waking state because brain uptake of the radiotracer was completed within 1 to 2 minutes after the injection.

SPM analysis of $^{99m}$Tc-ECD SPECT

In patients with RLS, SPM results demonstrated brain re-

Fig. 1. A Statistical Brain Map showing brain regions with decreased regional cerebral blood flow (rCBF) in patients with restless leg syndrome (RLS) compared to normal controls. rCBF was reduced in patients with RLS at the level of uncorrected $p<0.005$ in right anterior insular cortex. The results were superimposed on the 2-D planes of the averaged magnetic resonance imaging template of normal controls which was normalized to Montreal Neurological Institute space. Scales in the colored bars are t scores. The left-hand sides of the images represent the left hemisphere of the brain (radiological views).
gions with rCBF decrease compared to normal controls. According to SPM overlaid on T1 MR images reduced rCBF was found in right putamen (x, y, z=10, 14, 8, t=3.66) and right insular cortex (x, y, z=32, 24, -15, t=3.15) at the level of an uncorrected p<0.005 as shown in Fig. 1. There were no brain areas showing increased rCBF in RLS patients than normal controls. There were no significant correlations between rCBF and any clinical profile or PSG parameters in RLS patients and normal controls.

Discussion

We hypothesized that RLS patients with sleep onset and/or maintenance insomnia would have rCBF abnormality during wakefulness. The present study showed significantly decreased rCBF in the right putamen and right anterior insular cortex in RLS patients relative to normal controls.

The putamen and caudate together are called the striatum, which is the target of the cortical input to the basal ganglia. The most direct path in the motor loop through the basal ganglia originates with an excitatory connection from the cortex to cells in the putamen. RLS is known as a sensory-motor disorder. In large part because of the substantial dramatic symptomatic response to L-3,4-dihydroxyphenylalanine and dopaminergic agonist, a role for the dopaminergic system in the pathophysiology of RLS has been suggested. However brain imaging studies to reveal the relationship between dopamine and RLS have been inconsistent. These studies include PET and SPECT studies of dopamine-2/3 receptor binding potential, which have shown decreases, increases, and no change. An autopsy studies have found decreased dopamine-2/3 receptor in the putamen for RLS cases, which correlated negatively with RLS symptom severity. Previous SPECT studies of dopamine transporter in RLS have reported no differences from controls.

Although we used the radiotracer for assessing cerebral blood flow, significant change of putamen in patients suggested that putamen may be an important component of the motor circuit pathology in RLS.

The insular cortex is divided into two parts, the larger anterior insula and the smaller posterior insula in which more than a dozen field areas have been identified. The insula play a role in diverse functions usually linked to emotion or the regulation of the body’s homeostasis. These functions include perception, motor control, as well as cognitive functioning. The most typical clinical presentation of RLS patients is a pronounced discomfort or sensation, usually one or more of the limbs. Those sensory symptoms were described with various terms, suggesting all abnormal sensation of extremities. A study in patients with abnormal sensation after a lateral medullary infarct showed that simple stimulation such as light rubbing of the affected area induced both a pain sensation and brain activities which are usually associated with pain processing, notably in the thalamus, anterior insula, and posterior parietal cortex, while such activities were not observed when the same stimulus was applied to the normal side. The authors interpreted their findings as reflecting abnormal stimulus amplification in the thalamus and thalamo-parietal loops, leading to increased rCBF in the ‘lateral’ discriminative pain system (i.e., lateral thalamus and parietal cortex), and activating attentional networks.

Several lines of evidence, however, indirectly suggest that the putamen may contribute to the processing of sensory aspects of pain. The striatum is rich in opioid receptors and contains nociceptive neurons responsive to graded noxious stimuli. Nociceptive neurons in lamina V of the spinal cord project directly to globus pallidus, a structure that is intimately tied to the basal ganglia circuitry and the striatum. Additionally, striatal activation and striatal dopamine D2 receptor activity are correlated with individual variability in pain, pain modulation and several chronic pain syndromes. Recent functional MRI study showed that portions of the putamen activated during pain are connected not only with cortical regions involved in sensory-motor processing, but also regions involved in attention, memory and affect. Such a frame work may allow cognitive information to flow from these brain areas to the putamen where it may be used to influence how nociceptive information is processed. Tractography analyses performed in healthy subjects also showed that putamen was structurally connected with anterior insula and thalamus during nociceptive process.

Taken together, altered rCBF in putamen and anterior insular cortex may suggest the abnormal motor circuit and sensori-motor modulation of brains in RLS patients. It is not clear why the functional derangement of putamen and insular cortex persisted during wakefulness. It would be a more valuable if rCBF is compared between during daytime and during night time (when patients feel RLS symptoms) to find out the responsible neural substrate for the RLS symptoms.

Our study showed the cerebral dysfunction in RLS patients and these findings would provide the neuroimaging evidenc es responsible for the pathophysiology of idiopathic RLS.

REFERENCES

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