Correlation of Dopaminergic and Serotonergic Dysfunction in a Rat Model of Parkinson's Disease

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Correlation of Dopaminergic and Serotonergic Dysfunction in a Rat Model of Parkinson's Disease

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설레임과 두려움을 안고 시작했던 박사 과정을 마치며 지난 시간을 되돌아 봅니다. 저에게 대학원에서 훌륭하신 교수님들로부터 배움의 길을 열어주시고 졸업할 수 있도록 축복해주신 하나님께 감사와 영광을 돌립니다. 이 논문이 나오기까지 수많은 분들의 도움을 받았습니다. 그 분들의 도움이 없었다면 논문이 나올 수 없었을 것이기에 이 자리를 통해 감사의 인사를 드리고자 합니다.

먼저, 결실을 맺기까지 부족한 저를 믿어주시고 늘 많은 가르침과 기회를 주셨던 유영훈 교수님. 교수님을 향한 감사의 마음을 어떻게 글로 표현할 수 있을까요. 언제나 용기를 북돋워 주시던 교수님이 계셨기에 어려움도 잘 극복할 수 있었다고 말씀드리고 싶습니다.

본 논문의 처음 연구 계획에서부터 최종 마무리까지 동고동락하며 따뜻한 충고와 격려를 아끼지 않으셨던 최재용 박사님께도 깊은 감사의 인사를 드립니다. 박사님 덕분에 힘들었던 실험 과정도 막막했던 논문 작성도 끝까지 헤쳐나갈 수 있었습니다.

또한, 소동물 영상을 연구할 수 있도록 지원해주신 한국 원자력 의학원의 김경민, 최태현 박사님, 김병수 박사님, 어려운 환경에서도 성심

성의껏 연구를 도와준 서영범, 이치훈, 한상진 선생님께 머리 숙여 감사드립니다.

바쁘신 중에서 저의 논문심사를 맡아 주시고, 냉철한 시각과 혜안으로 조언을 아끼지 않으셨던 전태주 교수님, 석정호 교수님, 김철훈 교수님, 최준영 교수님께도 진심으로 감사드립니다.

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제 삶의 원동력이자, 항상 곁에서 힘이 되어주고 응원을 보내주는 남편과 아들 현도에게 감사의 인사를 전합니다. 오늘의 제가 있기까지 헌신적인 사랑을 베풀어주시고 언제나 신뢰를 보내주셨던 부모님, 며느리를 위해 기도와 응원을 아끼지 않으셨던 시부모님, 자랑스러운 동생 동훈이, 모두 감사드립니다. 우리 가족 모두와 이 작은 결실의 기쁨을 함께 나누고 싶습니다. 이 외에도 제가 미처 언급하지 못한 고마운 분들이 너무나 많습니다. 그 분들의 이름을 하나 하나 되새기지 못함을 죄송하게 생각하며, 이러한 도움이 더욱 빛나도록 앞으로도 최선을 다하겠습니다. 감사합니다.

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ABSTRACT

Correlation of Dopaminergic and Serotonergic Dysfunction in a Rat Model of Parkinson's Disease

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(Directed by Professor Young Hoon Ryu)

Depression frequently accompanies Parkinson's disease (PD) and has a great impact on patients' quality of life and disease progression. Previous research suggested that dopamine and serotonin systems are closely linked with depression

in PD. However, comprehensive studies about the relationship between these two neurotransmitter systems are limited. Therefore, the purpose of this study is to evaluate the effect of dopaminergic destruction on the serotonergic system in the same subject by using consecutive PET imaging. The interconnection between motor symptoms and depression was also examined.

[^{18}F]FP-CIT was applied to assess dopamine transporters and serotonin 1A (5-HT_{1A}) receptors were evaluated by [^{18}F]Mefway in unilateral 6-hydroxydopamine (6-OHDA) lesioned and sham operated rats. Behavioral tests were used to evaluate the severity of symptoms: rotational number for motor impairment and immobility time, acquired from the forced swim test for depression. Region-of-interests (ROIs) were drawn in the striatum and cerebellum for the dopamine system and hippocampus and cerebellum for the 5-HT system. Non-displaceable binding potential in the striatum and hippocampus were compared between 6-OHDA and sham groups.

As a result, unilateral 6-OHDA-lesioned rats exhibited significant bilateral reduction of hippocampal BP_{ND} for 5-HT_{1A} receptors compared with the sham group and there was a positive correlation between striatal BP_{ND} for DAT. The severity of motor symptoms was also closely related to the depression. Taken together, the data demonstrate that destruction of the dopaminergic system causes

the reduction of the serotonergic system and that the degrees of change in these neurotransmitter systems are also related with behavioral impairment in PD.

Keywords: 5-HT_{1A} receptor, PET, depression, Parkinson's disease

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I. INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder that is characterized by the loss of dopaminergic neurons in the striatum. Dopamine (DA) deficiency affects the cortical-basal ganglia circuit, which is mainly related to movement[1]. Therefore, the evaluation of the integrity of motor dysfunction is a

major issue for PD. Patients with PD, however, also suffer from non-motor symptoms (NMS) such as neuropsychiatric, autonomic and sensory symptoms, as well as sleep disorders. Depression is especially the most common neuropsychiatric co-morbidity in PD[2,3]. The prevalence of depression is reported in 20% to 90% of PD patients, which is higher than in healthy or other similarly disabled elderly control groups[4,5]. Depression in PD is important because it leads not only to poor prognostic influence but also has a great impact on patients' quality of life[6,7] .

Previous reports have shown that there is an inverse correlation between the severity of depression and dopamine transporter (DAT) availability in PD patients[8,9]. This supports that the nigrostriatal circuit is involved in PD-related depression. However, the pathophysiology of depression in PD may not be restricted to the dopaminergic system since dopamine replacement only partially improves depressive symptoms. The serotonin system is strongly implicated in mood changes, most notably depression, and multiple lines of evidence demonstrated altered serotonergic neurotransmission in PD[10-12]. Functional imaging studies for serotonin transporters also suggest that a malfunction of the serotonergic system is related to the occurrence of depression in PD patients[13,14]. However, there are few comprehensive studies about PD-related depression.

1. The current concept of Parkinson's disease.

The traditional concept that the first neuropathological insult leading to PD is the degeneration of dopaminergic neurons in the pars compacta of the substantia nigra (resulting in depleted levels of the dopamine) has been challenged. Parkinson's can no longer be considered a complex motor disorder characterized by extrapyramidal symptoms, but as a progressive multisystem disease with variegated neurological and non-motor deficiencies[15].

2. The non-motor symptoms of Parkinson's disease.

Non-motor symptoms (NMS) compromise symptoms such as mood changes, sleep problems, fatigue, autonomic dysfunction, gastrointestinal and sensory problems[16,17]. They represent a huge challenge to the treating physicians and allied health colleagues and continue to be a key determinant of patients' and caretakers' quality of life at huge societal costs[18,19]. In terms of the frequency and impact on the patients' and caretaker's quality of life and disease progression, the most troublesome NMS is depression[20].

A. The neurochemical basis of depression in Parkinson's disease.

(A) Dopaminergic dysfunction and depression in PD

Dysfunction of dopaminergic pathways evidently contributes to a range of non-motor symptoms in PD, thus highlighting the role of dopaminergic treatments to improve certain aspects of NMS[21].

In a previous report, there was an inverse correlation between the severity of anxiety and depression symptoms and left anterior putamen DAT availability in PD patients[22]. This means that there is an association between specific nigrostriatal dopamine system deficits and multiple neuropsychiatric symptoms in PD. This relationship may be mediated by impairment in frontal–subcortical circuits, as has been posited for PD-associated depression[23].

To date, there several clinical trials have investigated the efficacy of dopaminergic drugs on depression. However, they found that dopamine replacement only partially improved depressive symptoms. From these results, we can infer that the pathophysiology of depression in PD may not be restricted to the dopaminergic system.

(B) Serotonergic system and depression in PD

Generally, alteration of the serotonergic system is an important factor in the pathophysiology of the depression. Specifically, serotonin receptor subtype 1A ($5\text{-}HT_{1A}$) has been the most widely investigated and is known to play a central role in maintaining stable 5-HT transmission[24]. In the presynaptic site, they work as reuptake modulators of the synaptic 5-HT, causing a decrease in the release of 5-HT into terminal fields. $5\text{-}HT_{1A}$ receptors are also located postsynaptically in the cortical and limbic area[25-27].

The serotonergic (5-HT) system is strongly implicated in mood related-NMS of PD. Post-mortem, pathological, and functional imaging studies have demonstrated 5-HT signaling dysfunction in PD. In post-mortem studies of patients with PD, 5-HT depletion was observed in both the caudate and frontal cortex[10,28]. A pathological study revealed preferential loss of 5-HT in the caudate compared with the putamen but relatively low loss of 5-HT (66%) than dopamine (99%)[28-,29]. *In vivo* imaging studies have also demonstrated the depletion of 5-HT innervations to the striatum as measured by decreased 5-HT transporter binding[29, 30].

3. Positron emission tomography (PET)

PET is a valuable non-invasive imaging technique for studying both structural and functional connectivity. A major advantage of PET is its high sensitivity (10^{-9}-10^{-12} M) that allows for detection of the interactions between radioligands and their protein targets. This sensitivity is many orders of magnitude greater than the sensitivity with magnetic resonance imaging (MRI) (~10^{-4} M), or computed tomography (CT) (~10^{-3} M)[31,32]. In PD, PET can assist in the identification of dopamine deficiency and the evaluation of neurotransmitter and neuroreceptor function in vivo. PET serves as a biomarker and provides insights into both motor and non-motor symptoms of PD patients.

4. Purposes of research

The purpose of the present study is to investigate the relationship between the dopaminergic and serotonergic systems in the same subject, to assess an association between the severity of symptoms and depression in the animal model of PD.

II. MATERIALS AND METHODS

1. Animals

Seven male Sprague-Dawley (SD) rats (weighing 406.9 ± 34.5 g, mean ± SD) unilaterally lesioned with 6-hydroxy dopamine (6-OHDA; Sigma-Aldrich, St. Louis, MO, USA) were used. Lesioning protocol was performed as follows. Rats were pretreated with desipramine hydrochloride (12.5 mg/kg; intraperitoneal [i.p.] injection, 12.5 mg/ml, 0.1 ml/100g) before deep intraperitoneal ketamine/xylazine anesthesia (doses of 40 mg/kg and 5 mg/kg, respectively). Each animal's head was shaved and placed in a stereotaxic device (Stoelting Co., Wood Dale, IL, USA) and 6-OHDA (20 μg/4 μl/site, total of 40 μg 6-OHDA) was injected into two sites in the right striatum according to the rat brain atlas[33]. The injection sites (relative to the bregma and dura) were anterior-posterior (AP) +0.5 mm, medial-lateral (ML) 2.5 mm, dorsal-ventral (DV) −5.0 mm and AP −0.5 mm, ML 4.2 mm, DV-5.0 mm at a rate of 1 μl/min using a 26G Hamilton syringe. The inserted needle was withdrawn from each location after 5 min, and the wound was sutured. After 5 weeks, PET scanning was undertaken. All rats were housed in a temperature- and humidity-controlled room with a 12-h light/dark cycle and with free access to food and water. The exact same procedure was done for the seven control rats (sham group,

weighing 401.2 ± 15.5 g, mean ± SD), except that normal saline was administered to the striatum. These experimental protocols were approved by the committee for the care and use of laboratory animals of Yonsei University College of Medicine.

A. Mechanism of 6-OHDA for dopaminergic neuron destruction

The 6-OHDA is a hydroxylated analogue of the endogenous dopamine and relies on its specific uptake by the dopamine transporter (DAT) and noradrenaline transporter (NAT) from the extracellular membrane[34]. The toxin dose not cross the blood-brain barrier, and it is classically injected into intracerebral areas[35]. In the animal model of PD, desmethylimipramine (desipramine hydrochloride) was pretreated before administration of 6-OHDA to prevent uptake by noradrenergic terminals and to selectively destroy dopaminergic neurons. Intrastriatal injection of 6-OHDA induces prompt damage of striatal terminals and progressive cell loss of dopaminergic neurons[36]. The neuronal damage following striatal 6-OHDA infusion is mainly associated with massive oxidative stress caused by the toxin. Being similar to dopamine, 6-OHDA shows high affinity for the dopamine transporter. Once in the neuron, 6-OHDA accumulates in the cytosol and undergoes enzymatic degradation by monoamine oxidase-A (MAO-A) producing hydrogen peroxide, reactive oxygen species (ROS), and radicals as cytotoxic species[36]. In addition, 6-

OHDA can accumulate in the mitochondria, where it inhibits the activity of the electron transport chain by blocking complex I[37].

2. Assessment of rotational behavior: Striatum destruction

Two weeks after the 6-OHDA injections, contra- or ipsilateral rotational behaviors were induced by i.p. injections of D-amphetamine (5 mg/kg). A multichannel rotometer system (ROTORAT, MED Associates, Inc., St. Albans, VT, USA) was used for the evaluation of rotational behavior. Each animal was placed in a cylindrical test chamber for 60 min, and counter-clockwise rotations were analyzed. More than 100 rotations/min was considered to be evidence of successful lesioning and rats that met this criterion were used for further PET experiments.

To assess the severity of motor symptoms, the degree of striatal destruction was quantified by the rotation number of each rat. The association between motor symptoms and DAT availability was examined in the PD group.

A. Motor impairment associated with unilateral 6-OHDA injection

The 6-OHDA is commonly injected unilaterally in the striatum. Bilateral injection induced high mortality rate in the operated animals[38]. Rats with a

unilateral lesion of the striatum show rotational behavior in response to the administration of a dopamine agonist. They rotate ipsilaterally when injected with amphetamine, which acts as a presynaptic stimulator, by prompting the release of dopamine from striatal terminals, particularly on the intact side[39]. The rotational test has been the gold standard for 6-OHDA lesions, because it is objective, reliable, and clearly measures striatal dopamine receptor sensitivity[40].

3. Radioligand

[^{18}F]FP-CIT was used for DAT imaging and post-synaptic 5-HT_{1A} receptors were evaluated by [^{18}F]Mefway. [^{18}F]FP-CIT was obtained from Asan Medical Center, Korea and radiosynthesis of [^{18}F]Mefway was performed according to methods reported in previous studies[41,42].

A. Radioligand for imaging dopamine transporters

DAT is a plasma membrane protein expressed in dopamine neurons. It is involved in the regulation of extracellular levels of dopamine[43]. [^{18}F]-N-3-fluoropropyl-2b-carbomethoxy-3β-(4-iodophenyl)nortropane ([^{18}F]FP-CIT) is one of the DAT tracers most frequently used. It has an advantage that [^{18}F]Mefway is in

its use of ^{18}F. Labeling with ^{18}F allows for distribution to centers without a cyclotron, resulting in widespread clinical application. Additionally, the lower energy of positron provides higher intrinsic resolution[44]. DAT imaging has been widely applied to several central nervous system disorders. The main application has been the quantification of the dopaminergic deficit in PD.

B. Radioligands for imaging 5-HT_{1A} receptors

Many radioligands have been developed for imaging 5-HT_{1A} receptors and most of these tracers belong to the structural families of [*N*-(2-(1-(4-(2-methoxyphenyl)-piperazinyl)ethyl))-*N*-(2-(pyridinyl))cyclohexanecarboxamide] (WAY-100635), which is a selective and high-affinity 5-HT_{1A} receptor antagonist (K_D = 0.2 - 0.4 nM)[45-48]. Recently, Saigal et al. developed a very promising radioligand for human subjects, [^{18}F]Mefway, which has similar binding affinity and selectivity to 5-HT_{1A} receptors as [*carbonyl*-^{11}C]WAY-100635[48].

4. Acquisition of images

Each animal underwent two consecutive PET scans, using [^{18}F]FP-CIT and [^{18}F]Mefway with a 25 h interval (Figure 1). All brain PET images were acquired

with a Siemens Inveon small animal PET scanner (Siemens Medical Solutions, Malvern, PA, USA). After placing anesthetized rats in the gantry of the PET scanner, [^{18}F]FP-CIT was injected to the tail vein with an infusion pump for over 1 min. Tracer accumulation in the brain was investigated by a dynamic PET scan during 120 min. Animals had 24 hours of recovery time. Then, the rats were re-anesthetized and fluconazole (60 mg/kg, OneFlu injection, JW Pharmaceutical, Seoul, Korea) was given to suppress spillover of radioactivity from the skull[49] through the tail veins of the anesthetized rats with an infusion pump (Harvard pump 11 plus, Harvard Apparatus, USA) for 1 hour. Then, the rats were placed in the center of the PET scanner gantry. Emission data collection was started at the time of [^{18}F]Mefway injection, and radiotracer accumulation in the brain was examined by dynamic PET scans over 120 min. After emission, attenuation corrections were performed using data from a 10-min transmission scan with a ^{57}Co point source.

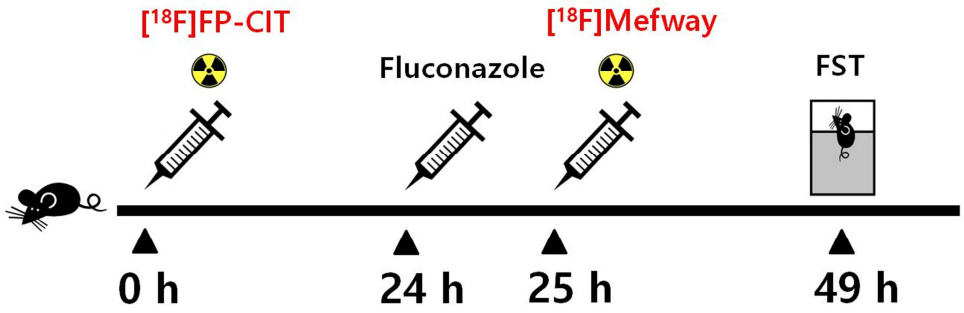

Figure 1. Time flow for the consecutive PET studies. After 24 hours of PET scan (duration 2 hours) with [^{18}F]FP-CIT, fluconazole was intravenously administrated for 1 hour. Then, [^{18}F]Mefway was injected, and PET scans were undertaken over 120 min.

A. Inhibition of [^{18}F]MEFWAY defluorination *in vivo*

[^{18}F]Mefway undergoes significant defluorination *in vivo* through the activation of cytochrome P450 2E1 (CYP2E1) isozyme[50,51]. This defluorination causes a spillover effect. The spillover effect means that radioactivity in the skull is also included in brain regions nearby, causing difficulties for the exact quantification of receptor density.

According to Choi et al.'s study, fluconazole showed a powerful suppression ability of [^{18}F]Mefway defluorination *in vivo*. Thus, fluconazole was given before [^{18}F]Mefway injection[49].

5. Image analysis

Raw list mode data were reconstructed in user-defined time frames (10 sec × 6 frames, 30 sec × 8 frames, 300 sec × 5 frames, 1800 sec × 3 frames) with voxel dimensions of 0.86 × 0.86 × 0.80 mm^3 by a 2-dimensional order-subset expectation maximization (OSEM) algorithm. The acquired images were evaluated by region-of-interests (ROIs) analysis via PMOD 3.1 software (PMOD Technologies Ltd., Adliswil, Switzerland).

For assessment of the dopamine transporters, volumes of interest (VOIs) were drawn in the striatum and cerebellum; then, time-activity curves (TACs) of [^{18}F]FP-CIT were acquired. Additional VOIs were drawn in the hippocampus and cerebellum followed by extraction of TACs using [^{18}F]Mefway. The cerebellum was chosen as a reference region for both radioligands because the region contains few dopamine transporters and 5-HT$_{1A}$ receptors[52,53]. Regional TACs were normalized in units of standardized uptake value (SUV) and calculated as

[(radioactivity in VOI in kBq/cc) x (body weight in kg)]/[injected dose in MBq] to normalize for the differences in weights and administered doses.

6. Kinetic analysis for binding values

We used the cerebellar cortex without the cerebellar vermis as reference tissue to obtain regional binding potentials. By using Ichise's multilinear reference tissue model (MRTM), an individual k_2' value was obtained from the TAC of the striatum or hippocampus with fixed time for the linearization (t*) at 40 min for both radioligands[54]. Then with these parameters, regional distribution volume ratios (DVR) were calculated by using the non-invasive Logan's graphical analysis method[55]. Estimated non-displaceable binding potentials (BP_{ND}) were calculated as the relationship: binding potential = DVR - 1.

7. Assessment of duration of immobility: The severity of depression

The forced swim test was used as an experimental tool for the evaluation of depressive moods. Rats rapidly cease attempts to escape and become immobile when forced to swim in a restricted space. The prolonged duration of immobility is often considered to be behavioral despair and to reflect a state of lowered mood in

the animal that can be extrapolated to a condition similar to human depression. Thus, it allows a correlation to be made between immobility and the neurotransmitter system[56].

After the [^{18}F]Mefway PET, awakened rats were placed in vertical tank (90 cm height x 20 cm in diameter) containing water (26^{o}C to a depth of 37 cm) for 15 min. After 24 hours, the rats were given a second forced swim test for 5 min and the motion of the rats were recorded by a video camera. These recordings were analyzed for the duration of immobility by commercial software (Smart, Panlab Harvard apparatus, Spain). The water was replaced between the tested rats to avoid the effects of excrement.

8. Statistical analysis

All data are presented as mean ± standard error of the mean (SEM). Differences in BP_{ND} values were analyzed with the Student's t-test. Pearson's correlation coefficient was used to determine relationships between DAT and 5-HT_{1A} receptors as well as motor and non-motor symptoms. All statistical analyses were performed with Prism 5 (ver. 5.04, GraphPad, La Jolla, CA, USA).

III. RESULTS

1. Effect of needle injury and confirmation of lesion

To exclude the effects of needle stick injuries, we compared the BP_{ND} for DAT in the striatum between the right and left striatum of sham-lesion controls. There was no statistical difference in BP_{ND} for DAT between the bilateral striatums of the sham group (Fig 2A). Successful lesioning was confirmed by comparing of the BP_{ND} for DAT between the lesion and intact striatum in the 6-OHDA lesioned rats. BP_{ND} for DAT in the lesioned striatum was significantly lower than that of the intact striatum ($p < 0.0001$, Figure 2B). There was no statistical difference in BP_{ND} for DAT of the intact striatum between the sham and 6-OHDA lesioned rats.

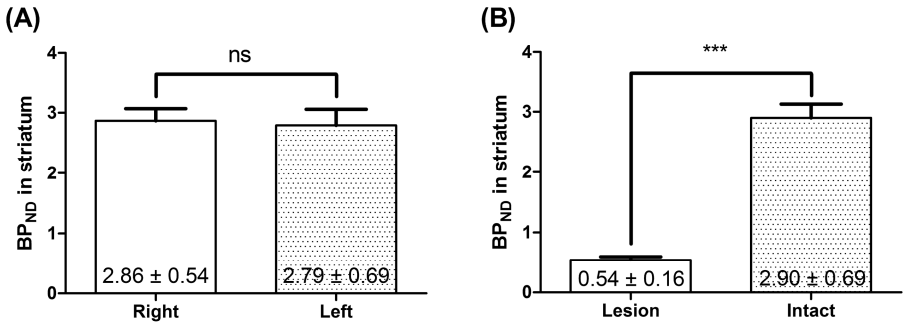

Figure 2. Comparison of [^{18}F]FP-CIT BP_{ND} in the sham-operated (A) and Parkinson's model (B). There were no significant differences between the right and left sites in sham group. There was significant reduction in the lesioned site ($^{***}p <$ 0.0004) compared with the right side of sham-operated rats. Data represent mean ± SEM values for four independent experiments.

2. BP_{ND} for DAT and severity of motor symptom in the Parkinson's model

The relationship between BP_{ND} for DAT in the striatum and the rotational number in 6-OHDA lesioned rats is illustrated in Figure 3. Rotation number showed an increasing tendency as BP_{ND} values for DAT decreased. A negative correlation

was found between the lesion side striatal BP_{ND} for DAT in 6-OHDA rats and motor impairment parameter (R^2= 0.79, p = 0.003).

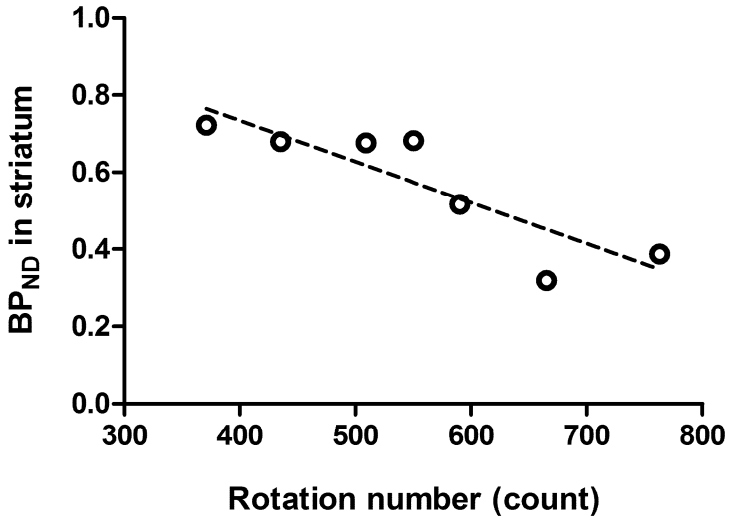

Figure 3. The relationship between the BP_{ND} for DAT and the severity of motor symptoms. As BP_{ND} for DAT decreased in the lesion side striatum, the rotational number showed a tendency to increase. Dashed line represents the linear regression (R^2= 0.79, p = 0.003).

3. Comparison of BP_{ND} for 5-HT_{1A} in sham and 6-OHDA lesioned group

To determine the effect of the destruction of DAT on the serotonergic system, we calculated the BP_{ND} of [^{18}F]Mefway in the hippocampus. BP_{ND} in the 6-OHDA lesioned group was approximately 22 % lower than the sham operated group with statistical significance (p = 0.028). In contrast there was no significant difference of BP_{ND} for 5-HT_{1A} between intact and lesioned striatums in each group (Figure 4).

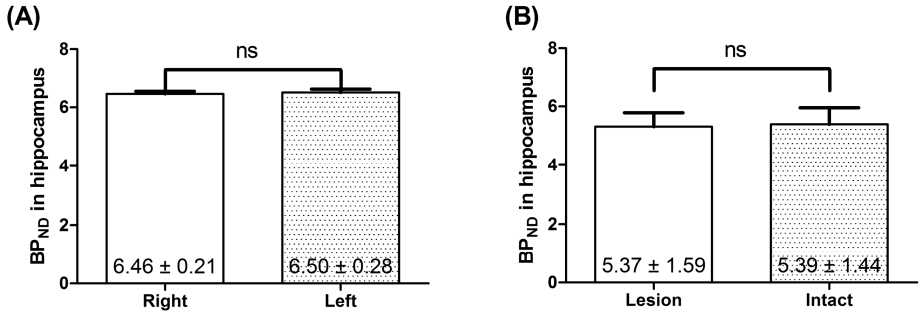

Figure 4. Comparison of [^{18}F]Mefway BP_{ND} in the sham-operated (A) and Parkinson's model (B). There were no significant differences between the lesion and intact sites in both groups. BP_{ND} value for the 6-OHDA lesioned group was lower for the sham group with statistical significance (p = 0.028). Data represent mean ± SEM values for four independent experiments.

4. BP_{ND} for 5-HT_{1A} and severity of depression

The relationship between BP_{ND} for 5-HT_{1A} and duration of immobility time in the 6-OHDA lesioned group is shown in Figure 5. The duration of immobility time showed a tendency to increase when the BP_{ND} for 5-HT_{1A} was low. A Pearson's correlation test revealed a negative correlation between the BP_{ND} for DAT in 6-OHDA and severity of depression (R^2= 0.81, p = 0.006).

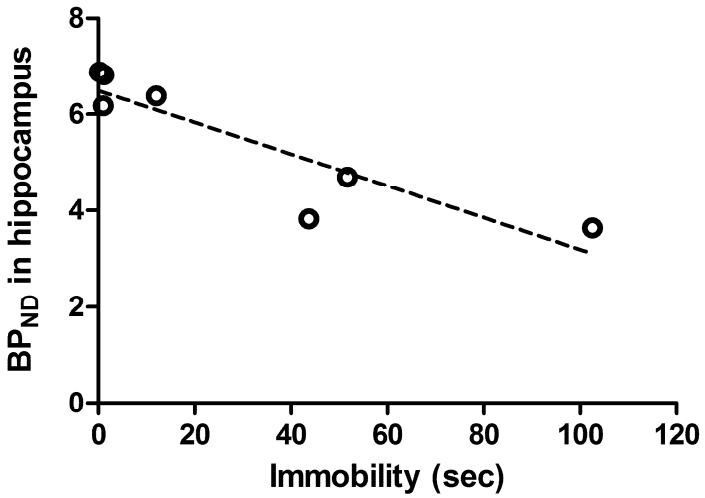

Figure 5. The relationship between BP_{ND} for 5-HT_{1A} and depression severity. As BP_{ND} for 5-HT_{1A} decreased, the duration of immobility time was prolonged.

5. The linkage between dopaminergic and serotonin systems

The association between dopaminergic and serotonin systems is demonstrated in Figure 6A. Correlation coefficients revealed a positive correlation between the hippocampal BP_{ND} for 5-HT_{1A} and the lesion side striatal BP_{ND} for DAT in 6-OHDA rats (R^2= 0.66, p = 0.026).

Additionally, 6-OHDA lesioned rats with severe motor impairment demonstrated longer immobility times. A positive correlation (R^2= 0.74, p = 0.014) was found between motor impairment and the severity of depression (Figure 6B).

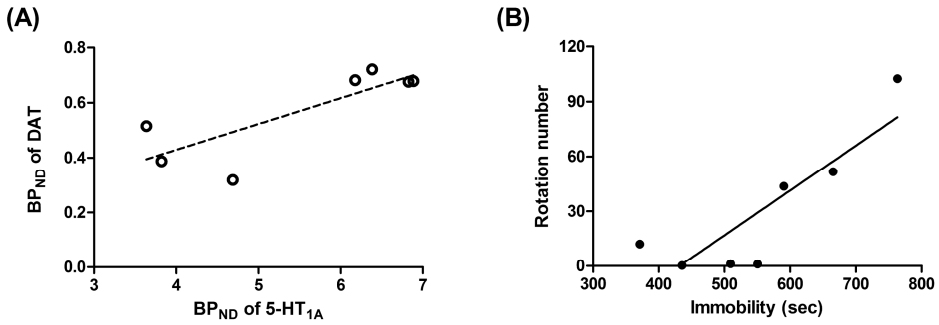

Figure 6. The relationship for BP_{ND} values between DAT and 5-HT_{1A} receptor (A): A positive correlation was noted between the hippocampal BP_{ND} for 5-HT_{1A} and the lesion side striatal BP_{ND} for DAT in the 6-OHDA model. The relationship between

severity of motor and depression symptoms (B): As the rotational number increased, the immobility time showed a tendency to be prolonged in the PD model.

IV. DISCUSSION

This was the first non-invasive neuroimaging study of the relationship between dopaminergic destruction and the serotonergic system in PD by means of PET and behavioral tests. Dopaminergic impairment is positively correlated with the alteration of the serotonergic system. The severity of motor symptoms also showed a close relationship to non-motor symptoms. These results can be extrapolated to a condition similar to behavioral impairment in human PD. Thus, results of this study may help further the understanding of the pathophysiology for depression in PD.

The HP was selected as an ROI in this study because it is known as a major target of 5-HT afferents within the limbic system. The high density of 5-HT receptors in the HP[57-59] indicates that it plays an important role in mood modulation[60]. For example, several magnetic resonance imaging studies demonstrated reduced HP volumes in individuals with depression[61-63]. A PET study of depressive patients who received selective 5-HT reuptake inhibitors showed metabolic activity changes in the corticolimbic circuits, including the HP[64].

In the present study, unilateral 6-OHDA lesioned rats were used to evaluate PD-related depression because 6-OHDA selectively destroys the presynaptic nerve endings of dopaminergic neurons and causes motor impairment in rats[65,66]. In addition, many researchers have already demonstrated that lesions of

dopaminergic neurons induced depression-like behaviors[67-69]. Hypo-dopaminergic and hypo–noradrenergic states were often observed in PD. Previous studies focused mainly on the dopaminergic system because the cardinal pathological characteristic of PD is degeneration of the dopaminergic neurons. However, the deficiency of dopamine dose not seem to be the sole cause of the dysfunction since its replacement only partially ameliorates PD symptoms such as tremor, bradykinesia, muscle rigidity and impaired posture[9,70]. In addition, the symptoms of PD appeared when about 70% of the dopamine-producing cells were damaged[71]. In light of these findings, the evaluation of the serotonergic system in PD has gained interest since pathologic, biochemical, and imaging studies indicate a loss of serotonergic neurons in PD[72]. Multiple lines of evidence suggested a diminished availability of serotonin transporters (SERT) in PD. However, the reduction of SERT was observed in non-depressed patients[30]. Therefore, we focused the integrity of 5-HT_{1A} receptors instead of SERT as a marker of serotonergic system changes.

It is interesting to note that the unilateral destruction of dopamine neurons generated bilateral changes in 5-HT_{1A} receptor uptakes. This result is in accordance with previous autoradiographic studies. Li and colleagues demonstrated that unilateral 6-OHDA lesions evoke bilateral down-regulation of cortical 5-HT_{2A} receptors[73]. Collectively, these findings indicate that 5-HT hypoinnervation in the limbic system can occur after 6-OHDA lesioning. There are several possible

explanations for the bilateral attenuation of the serotonergic system after destruction of the dopaminergic system.

The first is the reciprocal interaction between dopamine and serotonin[74]. Dopamine activates the serotonin neuron and serotonin inhibits the dopamine neuron. Therefore, the deficiency of the dopamine neuron resulting from the administration of neurotoxin might cause an inhibition of the serotonin neuron, reducing 5-HT in the synapse.

Second, the intervention of a glucocorticoid hormone-mainly cortisol-in humans and corticosteriod in rodents is associated with depression. Past research shows an elevation in circulating cortisol in PD patients and an increase in plasma corticosterone level in 6-OHDA lesion rats[75,76]. Diverse stressors activate a wide-spectrum of interacting neuroendocrine systems. In mammals, the limbic-hypothalamic-pituitary-adrenal (LHPA) system is a key neuroendocrine component of the stress response[77]. Neurons in the paraventricular nucleus (PVN) of the hypothalamus release a corticotrophin-releasing factor (CRF). The CRF stimulates the synthesis and secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary. ACTH then stimulates the glucocorticoid-producing adrenal cortex. As a result, LHPA elevates serum glucocorticoid level after stressors. Increased glucocorticoids cause neuronal damage to the hippocampus[78-81]. The decreased hippocampal volume suggests a mechanism for putative neuronal loss

seen within depressive patients either by apoptosis or inhibition of neurogenesis[82-84]. Other mechanisms are, however, also possible, such as reduction of the volume of individual neurons or reduction of glia tissue[85]. Moreover, glucocorticoid inhibits the activity of the tryptophane hydroxylase (TPH)[86]. 5-HT_{1A} receptors are highly concentrated in the hippocampus[81] and serotonin is biosynthesized from L-tryptophan via TPH and amino acid decarboxylase[86,87]. Suppressed TPH activity makes serotonin concentration decrease followed by a down-regulation of the 5-HT_{1A} in the plasma membrane, Thus, stressors may induce the demolition of hippocampal 5-HT_{1A} receptors and reduce 5-HT_{1A} receptor density.

A third possibility is involvement of habenula nucleus. Located at the thalamus, this highly conserved structure can be divided into the medial (MHb) and lateral habenula (LHb). The habenula receives inputs from the limbic system and basal ganglia[88]. LHb neurons project bilaterally to the SNc and ventral tegmental area (VTA), which are involved in the release of dopamine and 5-HT. It also projects to the median raphe nucleus (MRN) and dorsal raphe nucleus (DRN), which are also involved in 5-HT release. Efferent projections from the LHb terminate in variable structures, including nuclei of monoaminergic neurons; thus, it influences monoaminergic system modulation[89]. The habenula has been shown to be hyperactive in patients with major depression[90]. Based on this concept, Sourani et al. hypothesized that increased excitability of the internal globus pallidus in PD

leads to enhanced excitability of the LHb, which then causes a down-regulation of the 5-HT system because efferent projections from the LHb to DRN are inhibitory[91].

Fourth, the 5-HT hypothesis of depression in PD may explain our findings. Mayeux suggested that decreased 5-hydroxyindoleacetic acid (5-HIAA) levels in cerebrospinal fluid (CSF) in PD patients were associated with a PD subtype characterized by susceptibility to depression[92]. Campanella et al. showed that levodopa treatment causes decreased 5-HIAA CSF levels and depressive symptoms in PD patients[93,94.] In addition, post-mortem studies of PD human brains revealed decreased striatal 5-HT and 5-HIAA. The authors of these reports suggested that 5-HT cell loss in the raphe nuclei occurs prior to dopaminergic cell death[10,12,95]. Moreover, 5-HT_{1A} receptor adaptation will occur in the case of diminished synaptic levels of 5-HT. Previous studies using a chronic Parkinsonian monkey and a 6-OHDA lesioned rat model revealed 5-HT neuron loss in the dorsal raphe nuclei (DRN) and decreased 5-HT content in the hippocampus[96,97] (Frechilla et al., 2001; Wang et al., 2009). These results imply a decrease in total 5-HT innervation. In this state, post-synaptic 5-HT_{1A} receptor density is influenced by the reduction in innervation[98]. In the early phase following lesioning, post-synaptic 5-HT_{1A} receptors become hypersensitive to compensate for the loss of 5-HT[99]. In the chronic phase, these receptors will be internalized. We found a reduction of BP_{ND} in the lesioned group, indicating that post-synaptic 5-HT_{1A} receptor density was significantly

reduced at 5 weeks after 6-OHDA lesioning. Further research will be needed to demonstrate receptor density alterations using an early phase lesion in the PD model.

Fifth, depressive mood of 6-OHDA rats itself can be a cause of depression. Behavioral studies of a 6-OHDA-lesioned rat using the forced swim test, a widely used method for assessing depression-like behavior in rodents, demonstrated shorter latency and longer duration of floating, suggesting that striatal lesions induce depressive mood[68,69,100,101].

V. CONCLUSION

The present study demonstrated that the destruction of the dopaminergic system causes the reduction of the serotonergic system. There was a positive correlation between dopaminergic deficit and serotonergic impairment in terms of binding potential. Degrees of change in these neurotransmitter systems were also related with the severity of motor and depression symptoms. Our data confirm the existence of a link between dopaminergic and serotonergic systems and support the crucial role of these neurotransmitters in modulating depression in PD. Confirmation of these findings may improve the understanding of the pathophysiology of depression in PD and will lead to the development of adequate replacement strategies for the treatment of PD patients with depression.

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ABSTRACT (IN KOREAN)

설치류의 파킨슨병 모델에서 도파민계 및 세로토닌계 장애의 상호 연관성

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이민경

우울증은 파킨슨병에 흔히 동반되며, 환자의 삶의 질과 병의 진행에 많은 영향을 끼치는 것으로 알려져 있다. 이전 연구 결과들을 토대로 보면, 도파민계와 세로토닌계는 파킨슨병에 동반된 우울증과 밀접한 연관이 있는 것으로 여겨진다. 하지만, 아직까지 이 두 시스템

사이의 연관성에 중점을 둔, 통합 연구는 많지 않다. 따라서 이번 연구의 목적은 한 개체 내에서 도파민계의 소실에 따른 세로 토닌계의 변화를 PET 영상을 통해 분석하고자 하였다. 더불어, 운동 합병증과 우울증의 상호 연관성 또한 알아보았다.

파킨슨 병에 대한 모델로는 편측 선조체에 6-OHDA 를 투여한 랫트(unilateral 6-OHDA rat)를 사용하였고, 대조군으로는 편측 선조체에 생리 식염수를 주사한 랫트가 사용되었다. 두 그룹에서 [^{18}F]FP-CIT 는 도파민 운반체 영상화에 적용하였으며, [^{18}F]Mefway 를 5-HT$_{1A}$ 수용체는 영상화에 사용하였다. 운동 합병증의 정도는 도파민 효능 약물 투여로 발현된 회전 운동으로 평가하였고, 우울증의 심각도는 강제 수영 부하 시험(forced swim test)에서 부동 시간을 측정하여 평가하였다. PET 영상을 분석함에 있어 도파민계 영상에서는 선조체와 소뇌를, 세로토닌계 영상에서는 해마와 소뇌를 관심 영역으로 설정하였다. 이후, 6-OHDA 랫트 그룹과 대조군 사이에 해마 및 선조체의 결합능 차이를 비교하였다.

결과적으로, 편측 6-OHDA 랫트에서 해마의 5-HT$_{1A}$ 수용체 결합능이 대조군 대비 양측에서 현저하게 감소하였고, 선조체의 도파민 운반체의 결합능과 해마의 5-HT$_{1A}$ 수용체 결합능 사이에는 양의 상관 관계가 있음을 확인할 수 있었다. 또한, 운동 합병증의 정도와 우울증의 심각도 역시 밀접한 연관성을 보였다.

이를 종합해 볼 때, 연구의 결과는 도파민계의 소실이 세로토닌계의 감소를 야기한다는 것과 이 두 신경전달시스템의 변화 정도가 파킨슨병 환자의 운동 합병증 및 우울증의 심각도와 연관되어 있음을 시사한다.

핵심되는 말: 5-HT_{1A} 수용체, PET, 우울증, 파킨슨 병