L-DOPA-induced dyskinesia in a mouse model for Parkinson's disease produced by AAV vector-mediated overexpression of alpha-synuclein in midbrain dopaminergic neurons

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I dedicate this thesis to my wife, children, and parents who always supports and prays for me.

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ABSTRACT

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L-3,4-dihydroxyphenylalanin (L-DOPA) is the most effective drug in Parkinson's disease (PD), but long-term L-DOPA therapy leads to an abnormal involuntary movements termed L-DOPA-induced dyskinesia (LID). Rodent model induced by 6-hydroxydopamine (6-OHDA) was used for studies for LID, however it failed to reproduce the pathological hallmarks of PD. A new rodent model using adeno-associated virus (AAV) vector-mediated overexpression of alpha-synuclein was introduced and it displayed alpha-synuclein aggregation and progressive loss of dopaminergic neuron. The purpose of present study is to provoke LID in parkinsonian mice produced by AAV vector-mediated overexpression of alpha-synuclein and to explore histologic features in dopaminergic and serotonergic neuronal activities. C57BL/6 mice were injected with AAV2/7 vector including human alpha-synuclein transgene in substantia nigra. Eight weeks later, 10mg/kg of L-DOPA was injected daily for 7 days and 25mg/kg of L-DOPA was injected for following 7 days. Abnormal Involuntary Movements (AIM) scores were

rated for limb, axial, locomotive, and orolingual movements. The immunohistochemistry analyzed dopaminergic neuron loss in substantia nigra and degeneration of striatal dopaminergic and serotonergic fibers. LID was observed in two out of eleven mice at a daily dose of 10mg/kg L-DOPA and in all eleven mice at a daily dose of 25mg/kg L-DOPA. Tyrosine hydroxylase (TH)-positive nigral neuron in AAV vector-injected side was reduced by 25 to 70% of intact side, and optical density of striatal TH-positive fibers was reduced by 60 to 80%. The AIM scores were correlated with both TH-positive nigral neuron loss and optical density of striatal TH-positive fibers. Optical density of striatal serotonin transporter-positive fibers did not change. This study proved LID is produced in alpha-synuclein overexpressed mouse model. The results suggest that the role of dopaminergic and serotonergic activity in LID may differ between alpha-synuclein overexpressed and 6-OHDA-induced models.

Key words : L-DOPA, dyskinesia, Parkinson’s disease, adeno-associated virus, a-synuclein

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I. INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease following Alzheimer's disease. PD is characterized by motor symptoms including tremor, rigidity, bradykinesia, and postural instability. Pathological studies observed loss of dopaminergic neuron in substantia nigra (SN) and alpha-synuclein-rich cytoplasmic inclusions in the remaining neuron.[1]

L-3,4-dihydroxyphenylalanin (L-DOPA) is the most effective drug in dopamine replacement therapy, but long-term L-DOPA therapy leads to a choreiform abnormal involuntary movement termed L-DOPA-induced dyskinesia (LID). It is one of the most disabling complications in patients with PD. About 50% of PD patients treated with L-DOPA for 5 years had encountered LID,[2-4] and eventually LID developed in 90% of patients during long term L-DOPA therapy.[5]

Animal studies for pathophysiology of LID were mainly carried out with rodent models

induced by 6-hydroxydopamine (6-OHDA) which leads to dopaminergic neuron loss. Mice and rats that were lesioned with 6-OHDA at SN, medial forebrain bundle (MFB), or striatum displayed LID consistently. However, the 6-OHDA lesioned model presents dopaminergic neuron loss but not alpha-synuclein aggregation or formation of Lewy bodies which are the characteristic findings of PD pathology.[6] Therefore, this model could not reflect the pathological changes surrounding nigrostriatal pathway subsequent to progressive loss of dopaminergic neuron in human PD. Recently, a new rodent model induced by overexpression of alpha-synuclein using adeno-associated virus (AAV) vector was introduced.[7-10] Transduction of alpha-synuclein gene via AAV vector to the nigral dopaminergic neuron successfully caused dopaminergic neuron loss, alpha-synuclein aggregation in the remnant neuron, and hemiparkinsonism in behavioral tests. Since the pathological change and behavioral impairment were progressive, this model is considered to be more closely mimicking the pathological condition of human PD. LID is exclusively observed in PD and closely associated with neuropathological changes of PD, therefore histological characteristics of animal model by overexpression of alpha-synuclein may resemble the pathophysiology of the LID in patients with PD.

To date, study provoking LID in the rodent model induced by overexpression of alpha-synuclein using AAV vector have not been performed. In this study, we administered L-DOPA to parkinsonian mice injected with alpha-synuclein-encoding recombinant AAV2/7 vector, and performed histologic analysis for dopaminergic and seroternergic degeneration.

II. EXPERIMENTAL PROCESS

1. Animals

Male wildtype C57BL/6 mice (8 weeks old, 20–25g weight) were housed in cages under the 12:12-h light/dark cycle and temperature 23 °C. Mice accessed freely to food and water in the cages. All animal experiments were performed according to the institutional ethics review committee for animal experiment.

2. Experimental design

The experimental design is outlined in Figure 1. Firstly, all mice received an injection of AAV vector in right SN. Asymmetry was assessed by cylinder test at 1, 4, and 8 weeks after surgery. Eight weeks following surgery, mice were treated daily with L-DOPA for 14 days. The daily dose of L-DOPA was 10mg/kg for the first 7 days and 25mg/kg for the following 7 days. LID was rated right after L-DOPA injection at days 1, 7, and 14 of the treatment period. Three hours after the final LID scoring, animals were sacrificed and the brains were removed for immunohistochemistry.

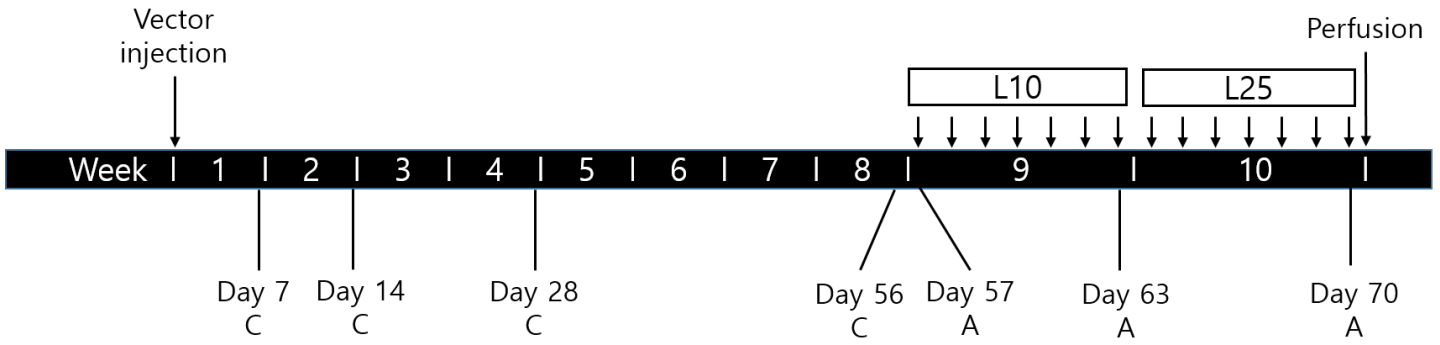

Figure 1. Experimental designs of the study. After AAV vector injection, cylinder tests (C) were performed according to the schedule. A daily 10mg/kg of L-DOPA (L10) was administrated at 9th week and a daily 25mg/kg of L-DOPA (L25) was administrated at the following week. Abnormal involuntary movement (A) was scored at the first day of L-DOPA injection and the last days of L10 and L25 phases.

3. AAV vector

Expression vector and packaging vector including the human wild type alpha-synuclein transgene were co-transfected into AAV 293 cells using calcium phosphate. The produced viruses were harvested 72 hours after transfection. Fluorescent expression of transduced cells were observed and photographed using fluorescence microscope. The final titer of the concentrated vector was 1.32E+13 genome copy (GC) per ml. The viral vector was produced at the Virus Facility of Korea Institute of Science and Technology (http://www.virus.kist.re.kr).

4. Stereotactic surgery

The animals were anesthetized with intraperitoneal injection of 0.4 ml of 1.25% 2,2,2-tribromoethanol (Sigma-Aldrich, St. Louis, MO, USA) solution and placed in a stereotactic frame (RWD Life Science, Shenzhen, China). 2 μl of AAV vector (4.0E+12 GC/ml) was injected in right SN at a rate of 0.25 μl/min with a 30-gauge needle on a 5 μl Hamilton syringe mounted in a microinjection pump. The needle was placed for additional 10 minutes and then withdrawn from the brain. Coordinates for the SN were determined according to a mouse brain atlas of Franklin and Paxinos[11]: anterioposterior (AP) -3.1 mm and mediolateral (ML) -1.2 mm from bregma, and dorsoventral (DV) -4.0 mm from the dural surface.

5. Behavioral analyses

Cylinder test

The asymmetry of forearm use was evaluated with cylinder test at 1, 2, 4, and 8 weeks from vector injection according to the previously described method.[12] The mice were

placed individually in an acryl cylinder (10.5 cm diameter, 25cm height) and video was recorded while the animal reached 20 weight-bearing touches on the cylinder wall. The numbers of the right and left touch were scored by an observer blinded to the lesion side of animals, and simultaneous both forelimb touches were excluded from the count.

L-DOPA administration and AIM rating

Eight weeks after vector injection, 10mg/kg L-DOPA methyl ester HCl and 12mg/kg benserazide HCl were administrated for 7 days with daily intraperitoneal injection. Then, 25mg/kg L-DOPA methyl ester HCl and 12mg/kg benserazide HCl were injected for days 8-14. On days 1, 7, and 14 of treatment, the AIMs were rated for 1 min every 20 min during 3-hour period from L-DOPA injection. AIM rating was performed by a blinded rater according to a previously described scale.[13-15] AIM was classified based on their topographic distribution into four subtypes: limb, repetitive myoclonic movement or dystonic posturing of the forelimb contralateral to the lesion; axial, lateral deviation or torsion of the head, neck and trunk towards the side contralateral to the lesion axial rotation of the neck; locomotive, circular locomotion with contralateral side; orolingual, repetitive chewing movement, facial grimacing, or tongue dyskinesia. Severity scores ranged 0–4 were given for the each as following: 0, absent; 1, occasionally, less than 50% of the observation time; 2, frequently, more than 50% of the observation time; 3, continuously, but interrupted by sensory stimuli; 4, continuously, not interrupted by sensory stimuli. The integrated AIM score was calculated as sum of the AIM score of nine observation times, thus the maximum integrated AIM score was 144.

6. Tissue preparation

At the end of last AIM rating, mice were anesthetized with 2,2,2-tribromoethanol (Sigma-Aldrich) and perfused transcardially with 50 ml saline followed by 50 ml of 4% paraformaldehyde. Brains were removed from mice and fixed in 4% paraformaldehyde for 24 hr and then transferred in 30% sucrose. Coronal 30-μm-thick sections through striatum and SN was cut using a cryomicrotome (Leica Microsystems, Bufallo Grove, IL) and collected in 6 regularly spaced series in cryoprotective solution (50% ethlylene glycol and 50% glycerin) and stored in -20 °C until use.

7. Immunohistochemistry

Free-floating sections were rinsed in PBS buffer and endogenous peroxidases were quenched with 3% hydrogen peroxide for 10 min. Non-specific binding sites were blocked by 5% normal goat serum in 0.25% Triton X-100 in PBS for 1 hr. Then, sections were incubated overnight at room temperature in anti-tyrosine hydroxylase (TH, rabbit polyclonal, 1:1000, AB152, Millipore, Burlington, MA, USA) and anti-serotonin transporter (SERT, rabbit polyclonal, 1:1000, AB9726, Millipore) with 0.25% Triton X-100 and 2% normal goat serum in PBS. After rinsing, sections were incubated in biotinylated secondary anti-rabbit antibody raised in mouse (1:200) for 1 hr. Tissue-bound primary antibodies were visualized by standard avidin-biotin-peroxidase method (Vectastain ABC kit, Vecta Laboratories, Burlingame, CA, USA) and 3,3'-diaminobenzidine (0.4mg/ml, Sigma-Aldrich) with 0.01% hydrogen peroxide. The sections were mounted, dehydrated, and cover-slipped.

8. Histological analyses

Stereologic quantification of TH-positive cells in SN pars compacta (SNc)

The total number of TH-positive cells in SNc were estimated using a stereologic quantification system with optical fractionator (StereoInvestigator, MicroBright-Field, Magdeburg, Germamy). A coronal section series (6 spaced) throughout rostro-caudal extent of the SNc (eight sections, -2.54 to -3.88 from bregma) were included for the count, SNc was delineated on each section according to the mouse brain atlas of Franklin and Paxinos.[11] Data are expressed as TH-positive cell in percentage of the intact side.

Estimation of optical densities (ODs) of TH-positive and SERT-positive fibers in striatum

Images of a section series (6 spaced) throughout rostro-caudal extent of the striatum (six sections, +1.10 to -0.10 mm from bregma) were obtained using a digital slide scanner (PANNORAMIC 250 Flash III, 3DHISTECH, Budapest, Hungary), and the ODs of TH-positive and SERT-positive fibers were measured using the ImageJ software (version 1.53, National Institute of Health, Bethesda, MD, USA). OD was adjusted for non-specific background density using the density on corpus callosum.[16] Data are expressed ODs of TH-positive or SERT-positive fibers in percentage of intact side.

9. Statistics

Pearson's correlation was used to reveal the relationship between AIM score and histological data (TH-positive cell count in SNc and ODs of TH-positive or SERT-positive fibers in striatum). Statistical analyses were performed using the GraphPad Prism Version 7 (GraphPad Software, La Jolla, CA, USA).

III. RESULTS

1. Cylinder test

To evaluate the asymmetry of forelimb use, the cylinder tests were performed at 1, 2, 4, and 8 weeks after vector injection. Contralateral (left) forelimb contacts were 46.9 ± 7.0% at 1 week and 43.8 ± 7.8% at 2 weeks after injection. Then, contacts of left forelimb were decreased to 33.6 ± 8.2% and 35.4 ± 12.7% at 4 and 8 weeks, respectively (Figure 2).

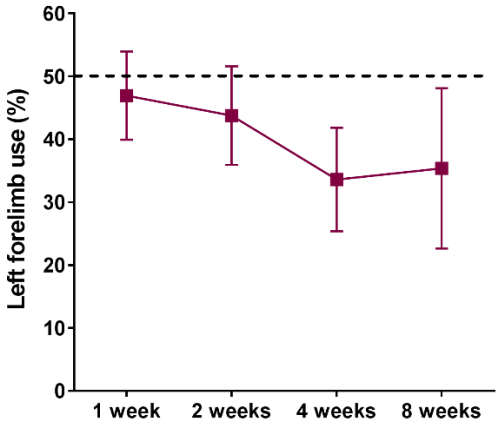

Figure 2. Asymmetric left forelimb use estimated by cylinder test. Left forelimb use was significantly decreased at 4 and 8 weeks.

2. Histological findings

TH-positive cell number in ipsilateral SNc to the AAV vector-injected side was reduced to 51.9 ± 12.6% (range, 30.3–74.8%) compared to contralateral intact side (Figure 3A, B). Accordingly, OD of TH-positive fibers in ipsilateral striatum was reduced to 29.1 ± 6.1% (range, 20.2–39.8%) compared to striatum on the contralateral side (Figure 3C, D). OD of SERT-positive fibers in ipsilateral striatum was comparable to that on contralateral side (102 ± 7.7%; range, 90.6–116.8%; Figure 3E, F).

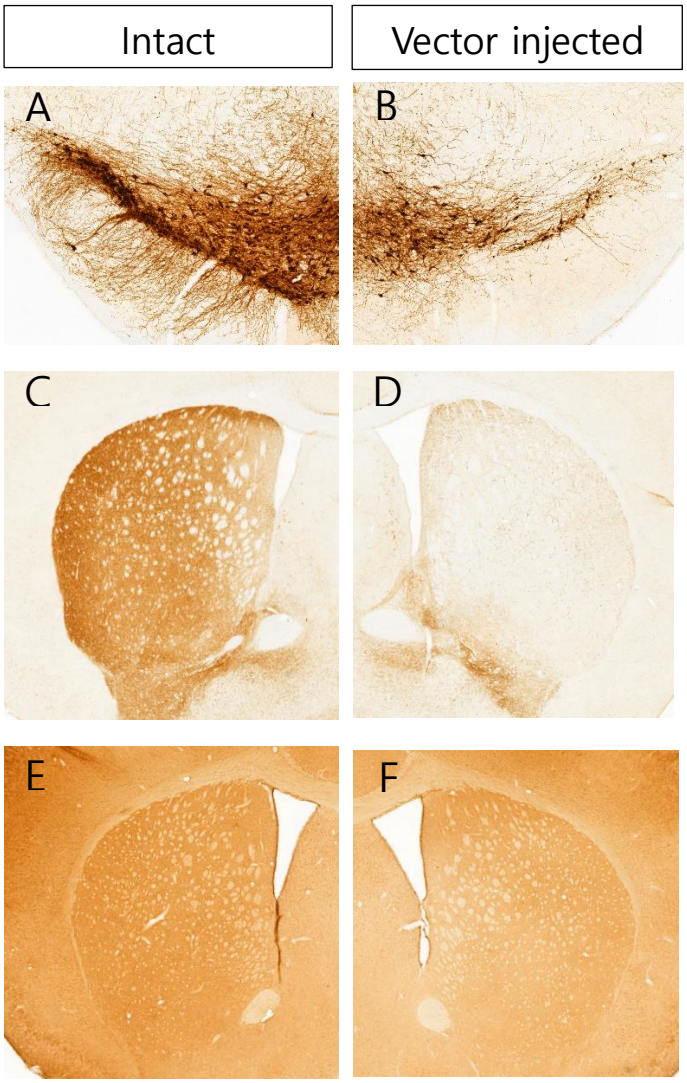

Figure 3. Immunohistochemical staining for tyrosine hydroxylase (TH) in substantia nigra (A, B), TH in striatum (C, D) and serotonin transporter (SERT) in striatum (E, F). TH-positive cell count in substantia nigra and optical density of TH-positive fibers in striatum were significantly lower in AAV vector-injected side (B, D) compared to intact side (A, C), however there was no difference between intact (E) and vector-injected (F) sides in optical density of SERT-positive fibers in striatum.

3. LID

AIM scores were evaluated at 1, 7, and 14 days from initiation of L-DOPA injection. At the first day when the first dose of 10mg/kg L-DOPA was injected, only 1 out of 11 mice exhibited dyskinesia (integrated AIM score 3, limb only; TH-positive cell count 32.2%; OD of striatal TH-positive fibers 20.2%; OD of striatal SERT-positive fibers 100.9%). At the 7th day when the last dose of 10mg/kg L-DOPA was injected, two mice showed dyskinesia: the mouse that dyskinesia occurred at the first dose (integrated AIM score 21, limb 10, axial 8, locomotive 3), and an additional mouse (integrated AIM score 2, limb only; TH-positive cell count 53.8%; OD of striatal TH-positive fibers 33.8%; OD of striatal SERT-positive fibers 98.2%). At the 14th day when the last dose of 25mg/kg L-DOPA was administrated, all of the eleven mice showed dyskinesia. The integrated AIM score was 21.9 ± 12.7 (range, 3–46) and dyskinetic movement was most prominent at 60 min after L-DOPA administration (Figure 4).

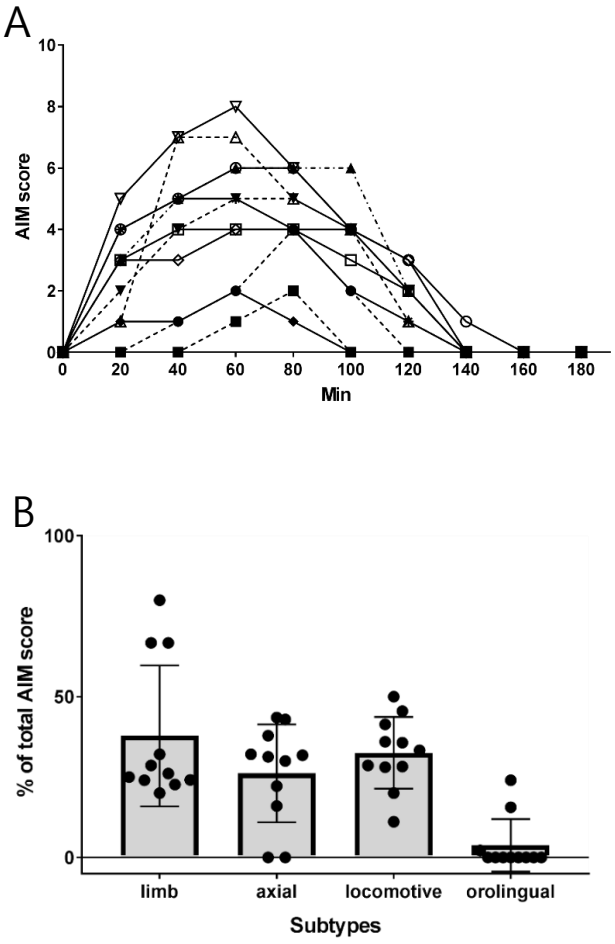

Figure 4. AIM score after injection of 25mg/kg of L-DOPA. (A) The AIM scores were highest at 60 minutes after injection. Each line represents an animal. (B) The proportion of limb, axial, and locomotive subtypes were comparable, but orolingual subtype had small portion.

4. Association between histological findings and LID

Integrated AIM score at the last dose of 25mg/kg L-DOPA (14th day) was used for this analysis. TH-positive cell count in SNc was negatively correlated with the integrated AIM score (R^2=0.57, p=0.007; Figure 5) and its subscores for limb (R^2=0.46, p=0.021) and axial movements (R^2=0.52, p=0.013). Subscores for locomotive and orolingual movements were not correlated with TH-positive cell count.

OD of striatal TH-positive fibers was negatively correlated with the integrated AIM score (R^2=0.67, p=0.002) and its subscores for limb (R^2=0.40, p=0.036), axial (R^2=0.61, p=0.005), and locomotive movements (R^2=0.66, p=0.002) but not orolingual dyskinesia (Figure 6).

There was not any association between OD of striatal SERT-positive fibers and integrated AIM scores as well as its subscores for limb, axial, locomotive, and orolingual movements (Figure 7).

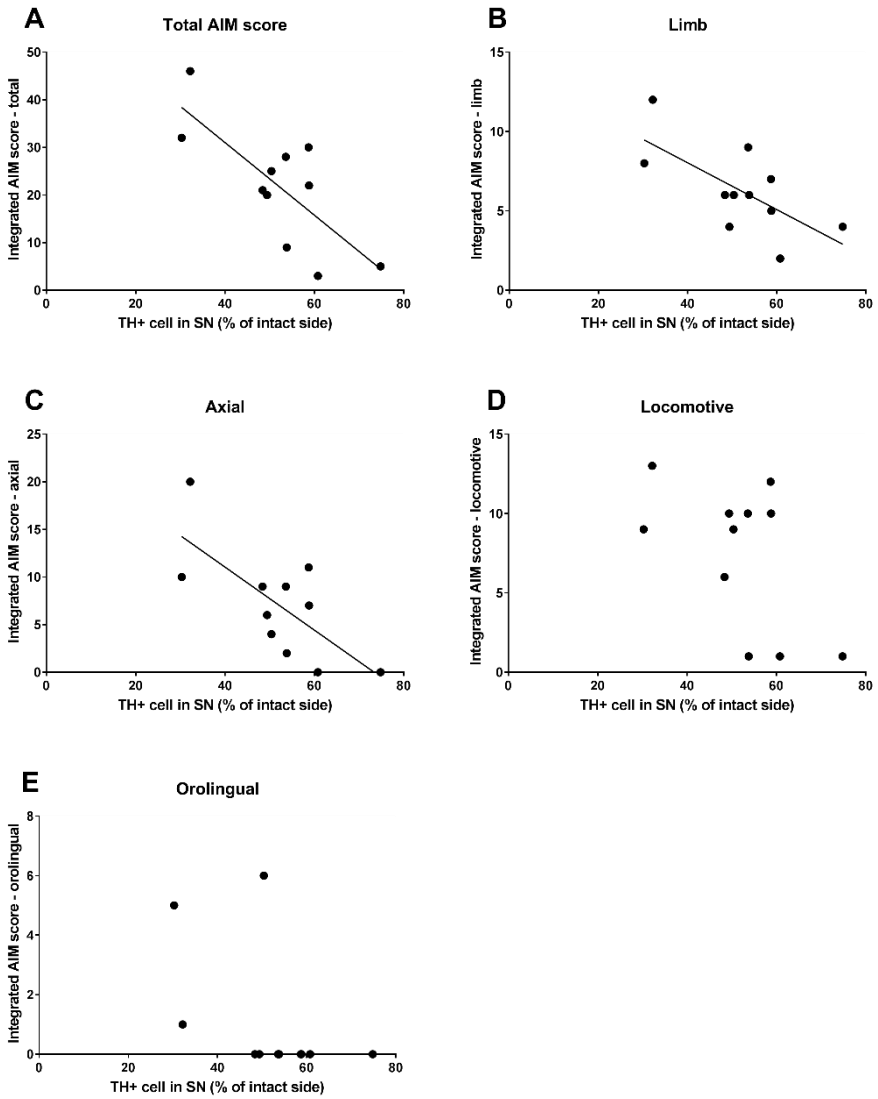

Figure 5. Association between tyrosine hydroxylase (TH)-positive nigral cell count and integrated AIM score. Integrated AIM score (A) and subscores for limb (B) and axial movement (C) were negatively correlated with TH-positive cell count, however subscores for locomotive (D) and orolingual movement (E) were not.

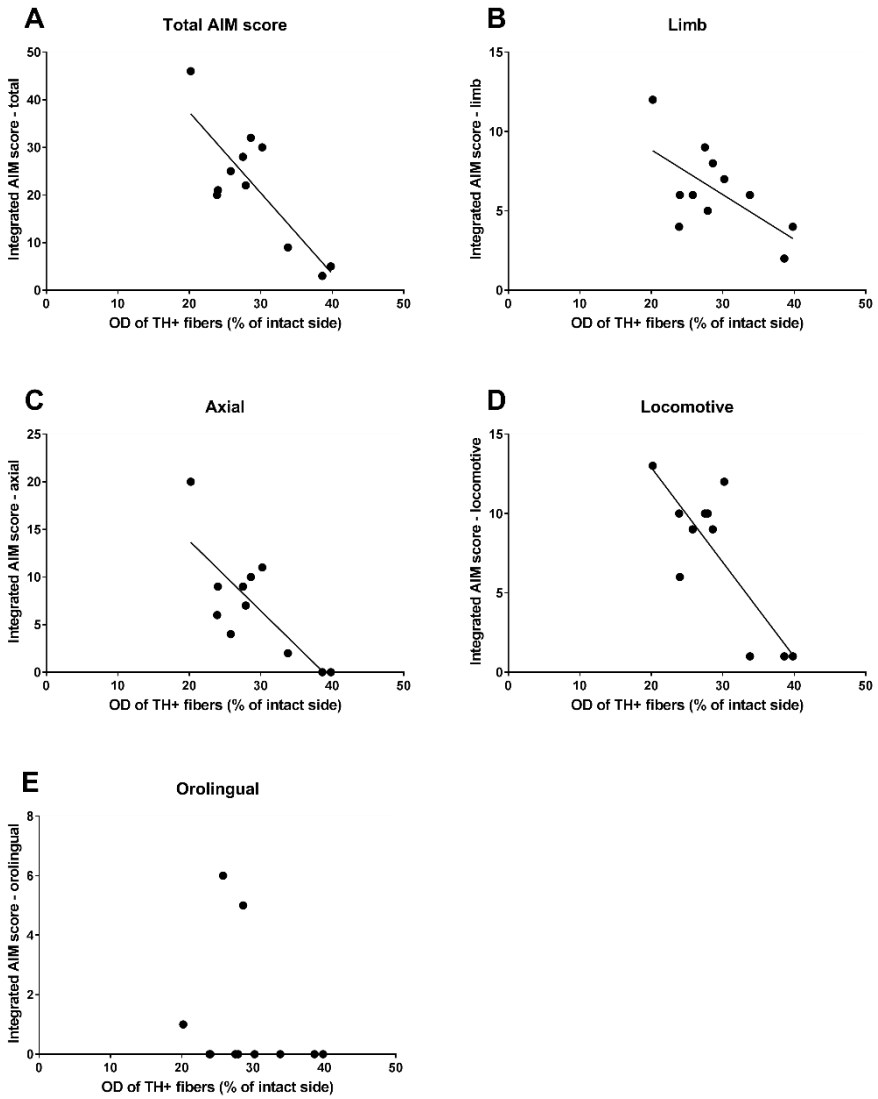

Figure 6. Association between optical density (OD) of striatal tyrosine hydroxylase (TH)-positive fibers and integrated AIM score. Integrated AIM score (A) and subscores for limb (B), axial (C), locomotive movement (D) were negatively correlated with OD of TH-positive fibers, however subscore for orolingual movment (E) was not correlated.

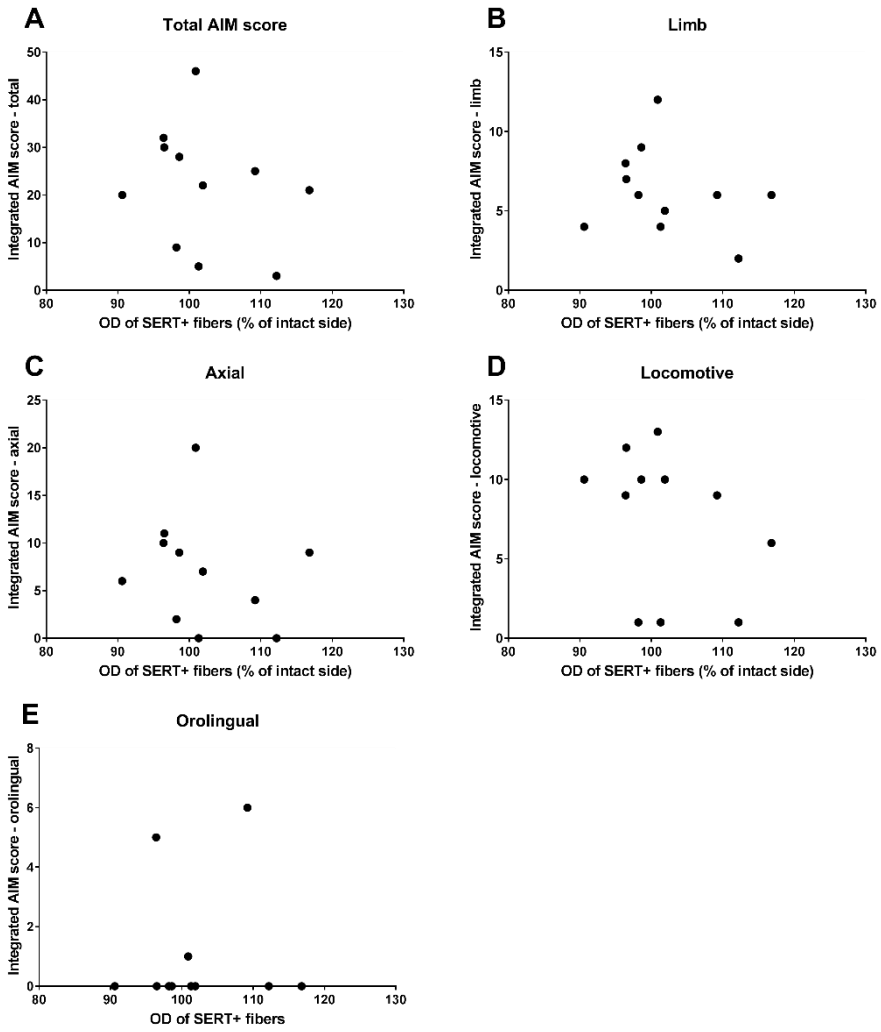

Figure 7. Association between optical density (OD) of striatal serotonin transporter (SERT)-positive fibers and integrated AIM score. OD of striatal SERT-positive fibers was not correlated with integrated AIM score (A) and its subscores (B–E).

IV. DISCUSSION

In this study, LID in a mouse model using AAV vector-mediated overexpression of alpha-synuclein was demonstrated for the first time. Rodent model induced by overexpression of alpha-synuclein displays pathological hallmarks of PD and progressive course in dopaminergic denervation. Therefore, histologic characteristics in present study may resemble those of PD patients with LID more closely.

Nigral dopaminergic neuron loss and striatal dopaminergic fiber degeneration play important roles in occurrence of LID. Several studies suggested that massive dopaminergic depletion is necessary to occurrence of LID, but results from previous studies are conflicting. A study of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned monkey observed LID in only animal with ≥95% striatal dopamine loss,[17] and another study in MPTP-lesioned marmoset also reported that >85% of striatal dopamine loss is necessary to occurrence of LID.[18] In the case of 6-OHDA-treated rat model, LID appeared in only animal with >70% striatal dopamine loss[19] or >80% SN cell loss.[20] However, LID also occurred in MPTP-lesioned squirrel monkeys having 40% nigral neuron loss and 60–70% striatal dopamine depletion[21] and in 6-OHDA-treated mice having 60–70% SN cell loss.[16] Studies are various in animal species, anatomical location of neurotoxin injection, area of measurement for dopaminergic depletion, L-DOPA dose, and treatment duration. In present study, LID was observed in all mice and SNc dopaminergic neuron loss and striatal dopaminergic fiber degeneration ranged from 25 to 70% (mean 38%) and from 60 to 80% (mean 71%) respectively. The extent of neuronal loss in SN was fairly milder than 6-OHDA-mediated model, but the striatal fiber degeneration was comparable to 6-OHDA model. Relatively lower extent of TH-positive cell loss than striatal TH-positive fiber loss was observed

repetitively in AAV vector-mediated rodent models,[7, 8] however the extent of cell loss and fiber loss were comparable in several studies.[9, 10, 22] The cardinal symptoms of human PD appear when about 30% of nigral dopaminergic neuron and 60–70% of striatal dopaminergic axon terminal were lost,[23, 24] and it is explained that axonal dysfunction precedes the nigral dopaminergic cell loss. This also supports that present model replicates the pathological progression of human PD. It is not clear which is the more relevant to development of LID, nigral cell loss or striatal axon degeneration. However, loss of buffering capacity is the key mechanism of LID occurrence,[25, 26] hence striatal axonal degeneration may be associated with LID more closely.

The dose of L-DOPA to induce dyskinesia varies in previous studies of mouse model produced by 6-OHDA. In the first study for LID in 6-OHDA-induced mice, a daily dose of 6mg/kg L-DOPA induced dyskinesia in MFB-lesioned mice, but a daily dose of 18mg/kg L-DOPA was required to induce comparable dyskinesia in mice received intrastriatal injection of 6-OHDA.[27] Another study in MFB-lesioned mice adopted a daily dose of 6mg/kg L-DOPA to produce LID.[28] An experiment in mice injected with 6-OHDA in SN, MFB, or striatum reported that significant LID occurred at a daily dose of 6mg/kg L-DOPA in MFB-lesioned mice but at 12mg/kg of L-DOPA in SN- and striatal lesioned mice.[16] Percentage of striatal dopamine loss was higher in MFB injection compared with striatal[16, 27] or SN injection,[16] therefore L-DOPA dose that required for LID onset was associated with extent of dopaminergic loss. In this study, LID occurred in two mice at 10mg/kg of L-DOPA dose and in all mice at 25mg/kg of L-DOPA. The extent of striatal TH-positive fiber loss was comparable or slightly mild to those in mice model induced by 6-OHDA injection in striatum[16, 27] or SN,[16] and the L-DOPA dose to induce dyskinesia of present study was also similar to the dose of (12–25mg/kg or 18–54mg/kg).

The OD of striatal SERT-positive fibers did not change in AAV vector injected side and there was no significant association between SERT fiber density and AIM score. Serotonergic activity is thought to play an important role in LID. Both destruction of serotonergic neurons by 5,7-dihydroxy-tryptamine and blockade of serotonergic neuron activity using combination of 5-HT1A and 5-HT1B receptor agonists suppressed LID near completely.[29, 30] Clinical studies also showed that eltoprazine (5-HT1A/1B receptor agonist) and buspirone (5-HT1A receptor agonist) attenuated the LID in patients with PD.[31, 32] However, it is unclear whether serotonergic innervation alters in LID occurrence. OD of striatal SERT-positive fibers in mice injected with 6-OHDA in MFB was increased, and it was higher in dyskinetic mice compared with non-dyskinetic mice.[28] In contrast, another study showed that OD of striatal 5-HT expression is slightly lower in mice injected with 6-OHDA in SN or striatum and significantly lower in mice injected in MFB.[16] The integrated AIM score was inversely correlated with decrement of 5-HT density in mice injected in MFB but not in mice injected in SN or striatum.[16] Serotonergic activity in patients with PD measured by functional neuroimaging was lower than healthy control or early drug-naïve patients, but the difference between patients with and without LID is unclear: lower in patients with LID than those without LID in a study,[33] and no difference in another studies.[31, 34] Two studies commonly reported that serotonin/dopamine binding potential ratio was significantly higher in patients with LID.[33, 34] It suggests that relative rather than absolute change in dopaminergic and serotonergic innervation is associated with occurrence of LID. In present study, the striatal SERT/TH ratio was significantly correlated with integrated AIM score (r=0.68, p=0.021), but it seems to be mainly due to TH variability. Further works may be required to assess the accurate role of serotonergic change for LID in alpha-synulein overexpression model.

V. CONCLUSION

Present study observed LID in a parkinsonian mouse model produced by AAV vector-mediated overexpression of alpha-synuclein for the first time. In this model, TH-positive neuron loss in SNc and TH-positive fiber degeneration in striatum was correlated with the severity of LID rated by AIM score, while the serotonergic activity did not change in dyskinetic animals. LID occurred in relatively mild dopaminergic loss (25–70% of SNc neuron loss and 60–80% of striatal fiber loss) than 6-OHDA-induced model and the dopaminergic involvement was more prominent in striatal axonal dysfunction than nigral cell loss. The results suggest that the role of dopaminergic and serotonergic activity in LID may differ between alpha-synuclein overexpressed and 6-OHDA-induced models.

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APPENDICES

< ABSTRACT (IN KOREAN)>

아데노연관바이러스 벡터를 이용하여 중뇌 도파민세포에서 알파시누클레인을 과발현시킨 파킨슨병 마우스 모델에서의 L-DOPA 유발 이상운동증

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홍진용

L-DOPA는 파킨슨병에서 가장 효과적인 약물이지만, 장기적인 L-DOPA 투약은 L-DOPA 유발 이상운동증이라는 비자발적 이상운동을 유발한다. L-DOPA 유발 이상운동증의 연구에는 주로 6-OHDA를 이용한 설치류 모델이 이용되었으나, 이 모델은 파킨슨병의 병리학적 특징을 보여주지 못하였다. 이에 최근에는 아데노연관바이러스를 이용한 알파시누클레인 과발현 파킨슨 모델들이 소개되었는데, 이 동물들에서는 알파시누클레인 응집과 진행성의 도파민 신경세포 손실이 관찰되었다. 본 연구에서는 이 파킨슨 마우스 모델에서 L-DOPA 유발 이상운동증을 발현시키고 도파민과 세로토닌 활성에 관한 조직학적 특성을 살펴보고자 하였다. C57BL/6 마우스의 흑색질에 사람 알파시

누클레인 유전자가 삽입되어 있는 아데노연관바이러스2/7형 벡터를 주사하였다. 8주 후 10mg/kg 농도의 L-DOPA를 7일간 매일 주사하였고, 다음 7일동안은 25mg/kg 농도의 L-DOPA를 매일 주사하였다. 이상운동증은 앞다리 움직임, 체축의 기움, 회전운동, 혀와 입의 이상운동 정도를 측정하였다. 면역조직화학염색을 통해 흑색질의 도파민 신경세포 손실 정도와 줄무늬체에서의 도파민성과 세로토닌성 신경섬유의 퇴행 정도를 측정하였다. 10mg/kg의 L-DOPA 농도에서는 11마리 중 2마리에서 L-DOPA 유발 이상운동증이 관찰되었고, 25mg/kg의 농도에서는 11마리 모두에서 L-DOPA 유발 이상운동증이 관찰되었다. 벡터를 주사한 쪽 흑색질의 tyrosine hydroxylase (TH) 양성 신경세포는 정상측에 비해 25-70% 정도 감소하였고, 줄무늬체 TH 양성 신경섬유의 광학밀도는 정상측에 비해 60-80% 정도 감소하였다. 이상운동증 점수는 흑색질의 TH 양성 세포 감소 정도, 줄무늬체 TH 양성 신경섬유 감소 정도와 모두 비례하였다. 하지만, 줄무늬체에 있는 세로토닌운반체 양성 신경섬유의 광학밀도는 변화가 없었다. 본 연구는 알파시누클레인 과발현 마우스 모델에서 L-DOPA 유발 이상운동증을 발생시킬 수 있음을 보여주였다. 또한 이 모델과 기존의 6-OHDA를 이용한 모델에서 도파민성과 세로토닌성 활성이 L-DOPA 유발 이상운동증에 미치는 영향이 다를 수 있음을 시사한다.

핵심되는 말 : L-DOPA, 이상운동증, 파킨슨병, 도파민, 세로토닌

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