의학박사 학위논문

신경 염증 소동물 모델에서 ^{18}F-fluorodeprenyl-D_2와 ^{18}F-THK5351의 MAO-B 활성도 측정 능력 평가

Evaluation of potential ability of ^{18}F-fluorodeprenyl-D_2 and ^{18}F-THK5351 for detection of MAO-B activity in a rat model of acute neuroinflammation

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이 논문을 의학박사 학위 논문으로 제출함

2020년 8월

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영문 요약

Background

In recent years, inflammatory processes have been found to be involved in the etiopathology of many neurological disorders. Being able to image neuroinflammation non-invasively in patients is critical to monitor pathological processes and potential therapies targeting neuroinflammation. To date, a range of novel radioligand tracers for glial cell have been developed to image the activations of microglia and astrocytes. Monoamine oxidase B (MAO-B) is one of the molecular targets to image astrocytes and has been expected to be a reliable neuroinflammatory biomarker for PET imaging. However, the potential ability for detection of MAO-B activity in neuroinflammatory process, has not been fully evaluated, especially in acute neuroinflammatory model. In this study, we evaluated potential ability of two MAO-B binding radiotracers, ^{18}F-fluorodeprenyl-D_2 and ^{18}F-THK5351, to visualize and quantify MAO-B activity that involves in acute neuroinflammatory process.

Materials and Methods

Adult SD rats were injected with lipopolysaccharide (LPS) intraperitoneally (i.p.;5-10mg/kg) and intracranially (i.c.;1ug or 10ug). After 16-24h of LPS injection, animals underwent 30 min dynamic PET/MRI scan with ^{18}F-THK5351 (n=4, for each group) or ^{18}F-fluorodeprenyl-D_2. (n=4, for each group). Time activity curve obtained expressed as standardized value (SUV) and uptake ratio (SUVR, reference: cerebellum) using region based VOIs. For quantification of tracer uptake, region based VOIs or fixed sized VOIs were placed at 10-30 summed image and compared with those of control rats or unaffected contralateral hemispheres. Ex vivo cytokine array assay and immunohistochemistry analysis were performed to confirm *in vivo* results.

Results

^{18}F-THK5351 and ^{18}F-fluorodeprenyl-D_2 crossed BBB, bound rapidly with constant washout from rat brain on baseline scan of control group. In intraperitoneal LPS injection group, ^{18}F-THK5351 uptake of cortex, subcortex and cerebellum increased without regional difference, when compared with control (1.17 ± 0.07 vs. 0.68 ± 0.05, $p < 0.001$); ^{18}F-fluorodeprenyl-D_2 uptake also increased, but there was no statistical significance (1.97 ± 0.06

vs. 1.81 ± 0.08). In intracranial LPS injection group, ^{18}F-THK5351 uptake increased in ipsilateral hemisphere at the LPS injection site, while ^{18}F-fluorodeprenyl-D_2 uptake did not (core/contralateral ratio = 1.16 ± 0.07 vs. 1.05 ± 0.08).

Conclusion

^{18}F-THK5351 showed increased retention in rat brain with acute neuroinflammation. But ^{18}F-fluorodeprenyl-D_2 did not show significantly increased retention. These results suggest that ^{18}F-fluorodeprenyl-D_2 might not be suitable for detection of acute neuroinflammation in rat brain. Further studies are needed to determine the specificity of ^{18}F-THK5351.

Key words: ^{18}F-THK5351, ^{18}F-fluorodeprenyl-D_2, monoamine oxidase, neuroinflammation, Positron emission tomography

차 례

표 차례

그림 차례

서론

In recent years, inflammatory processes have been found to be involved in the etiopathology of many neurological disorders as diverse as stoke, multiple sclerosis and neurodegenerative disease. In this neuroinflammation process, microglia and astrocytes are fundamental in defending the brain against infection and injury. Because glial cell activation not only reflects neuronal dysfunction but can also directly influence disease progression, considerable efforts have been made to develop non-invasive techniques to monitor these cells *in vivo*. To date, a range of novel radiotracers for glial cell imaging is under development and several radiotracers have been developed to image the activations of microglia and astrocytes in experimental models and in various CNS diseases using PET imaging (1).

Monoamine oxidase B (MAO-B) is one of the molecular targets to image astrocytes. It plays an important role in regulating chemical neurotransmitters by catalyzing oxidative deamination of, for example, monoamine neurotransmitters (2) and selectively oxidizes monoamines and generates hydrogen peroxide, which can react to form highly reactive oxygen species (3). Recent studies demonstrate upregulation of MAO-B on the outer membrane of astrocytic mitochondria that activated during neuroinflammatory process (4), thereby suggesting the potential for measuring alterations in MAO-B as a marker of reactive astrogliosis in neuroinflammation (1).

Several radiotracers have been developed for MAO-B PET imaging. Among few MAO-B PET tracer available, radiolabeled analogs of *L*-deprenyl (selegiline) have used for detection of reactive astrogliosis in neuroinflammation disorder diseases including Alzheimer disease (AD) (5-7), amyotrophic lateral sclerosis (8), and Creutzfeldt-Jakob disease (9). ^{18}F-flurodeprenyl-D_2 is a radiofluorated, bis-deuterium substituted *L*-deprenyl analog, improving MAO-B quantification through the deuterium isotope effect, which slowed the rate of irreversible binding to MAO-B (10,11). Indeed PET studies with ^{18}F-flurodeprenyl-D_2 revealed favorable kinetic properties with relatively fast washout from NHP brain and improved sensitivity for MAO-B imaging (10).

^{18}F-THK5351 is one of the potential radiotracers for monitoring MAO-B activation during neuroinflammatory process. ^{18}F-THK5351 is known as a quinoline-derivative tau imaging tracer with affinity to paired helical filaments, a typical tau aggregate present in neurofibrillary tangles. In addition to the affinity to tau protein, ^{18}F-THK5351 has also been

reported to bind to MAO-B, which is attributed to its off-target binding in the striatum, thalamus, and brainstem(12) . Binding to MAO-B in vivo in humans was recently demonstrated for ^{18}F-THK5351, in which selegiline (MAO-B inhibitor) reduced the cortical PET signal by 40% in patients with AD (13). ^{18}F-THK5351 devoid of affinity for MAO-B and expected to be used for monitoring the neuroinflammation in vivo. Up to date, the majority of preclinical and clinical studies have been using MAO-B tracers for assessing MAO-B activity in CNS system and investigating astrocytosis in neurodegenerative disease or models of chronic inflammation, in which the level of glial activation is much lower and slower and is thus far differ from those in acute inflammatory process (14,15). There is keen interest in the development of noninvasive diagnostic modality for detection of neuroinflammation in acute phase. Also, there is a need to set up an easy-to-perform, robust preclinical model for radiotracer development.

In this study, we induced acute neuroinflammation via intraperitoneal (i.p.) and intracranial (i.c.) injections of LPS in rats and set out to validate the MAO-B targeting radiotracers, ^{18}F-THK5351 and ^{18}F-fluorodeprenyl-D_2, as surrogate imaging biomarkers of astrogliosis. The purpose of this study was to evaluate the potential ability of ^{18}F-THK5351 and ^{18}F-fluorodeprenyl-D_2 in detection of MAO-B activity that involves the activations of the astrocytes in acute neuroinflammation.

연구대상 및 방법

Radiotracer production

^{18}F-THK5351 and ^{18}F-fluorodeprenyl-D_2 were synthesized as described previously (16). Fluorodeprenyl-D_2 and THK5351 were successfully labeled with ^{18}F and this was confirmed by radio-HPLC. The tracers were produced with high radiochemical purity, > 95%. The overall radiochemical yield and specific activity of ^{18}F-THK5351 were 33.4 ± 9.6% and 617 ± 121 MBq/mL, and those of ^{18}F-fluorodeprenyl-D_2 were 15.4 ± 2.9% and 594 ± 101 MBq/mL respectively, satisfying all quality control criteria.

Animals and treatments

The animal research protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the Asan Institute for Life Science (registration no. 2020-12-057). For the neuroinflammation model, SD rats (8-9 weeks old) were purchased from the JA Bio (Korea) and maintained in accordance with IACUC guidelines. The rats in each cage were divided into the following LPS (Sigma Aldrich, USA) treatment groups for each of the four experiments: (1) Control group, (2) intraperitoneal LPS (5 or 10 mg/kg) injection group (3) intracranial LPS (1 μg/μl) group, (4) intracranial LPS (10 μg/μl) group. Each group consisted of eight rats for PET imaging (four for ^{18}F-THK5351 and four for ^{18}F-fluorodeprenyl-D_2 PET imaging). Intracranial injections of LPS were conducted via the left postglenoid foramen as the method previously described by Iwami et al. with some modifications (17). After induction of anesthesia with isoflurane (1.5% isoflurane in 100% O2 gas), the rats were placed, and the appropriate angle and depth were adjusted for correct needle insertion. After 16~24 h LPS injection, rats underwent PET imaging.

Animal PET-MRI imaging

PET-MRI fused imaging was performed using a nanoScanPET/MRI system (1T, Mediso, Hungary). Continuing to keep the rat warm, administer intravenously via the tail vein 6.5 ± 1.0 MBq in 0.2 mL of ^{18}F-THK5351 or ^{18}F-fluorodeprenyl-D_2 and keep the rat under anesthesia MR brain imaging obtained T1 weighted with Gradient-echo(GRE) 3D sequence (TR = 15 ms, TEeff = 3, FOV= 64 mm, matrix = 128x128) and T2 weighted with Fast Spin Echo(FSE) 3D Sequence (TR= 2400 ms, TEeff = 110, FOV = 60 mm, matrix = 220x220)

images, which were acquired before the tracer injection. 30 minutes of dynamic PET images were acquired in a 1-3 coincident in a single field of view with MRI range. Body temperature was maintained with a heating air on the animal bed (Multicell, Mediso, Hungary) and a pressure sensitive pad was used for respiratory triggering. PET images were reconstructed by Tera-Tomo™ 3D, in full detector mode, with all the corrections on, high regularization and 8 iterations.

Image analysis

PET image analysis was performed using the InterView Fusion software package (Mediso, Hungary) and applying standard uptake value (SUV) analysis.

For the analysis of PET images of rats injected with LPS intraperitoneally, volume of interest (VOI) were delineated on co-registered MR/PET images, and the delineation was guided by referring to an atlas of rat brain. Five VOIs selected for evaluation as follow: striatum, cortex, thalamus, hippocampus, and cerebellum. Decay-corrected time activity curves for all regions plotted over time and radioactivity were expressed as standardized uptake value [SUVmean = (tumor radioactivity in the volume of interest with the unit of Bq/cc × body weight) divided by injected radioactivity). The cerebellum was used as a reference and the target to cerebellum ratio was calculated for each VOI (SUVR). For the semi-quantitative analysis of PET images of rats injected with intracranial LPS injection, a spherical VOI of fixed size (diameter of 3 mm) was positioned on summed image of 10-30 min of each experiment. Within the brain (delineated using MRI images) VOIs were then manually placed and labeled under the following scheme: (1) core of the LPS injected lesion (covering the region with highest uptake around the injection site), (2) contralateral region (mirrored core VOI in the contralateral hemisphere to act as a reference region).

Ex vivo cytokine array assay

To detect the levels of cytokines in LPS-induced inflammatory brain, antibody-based cytokine array system (C1, Raybiotech, USA) was used following the manufacturer's instructions. Briefly, brain tissues were lysed in 2 x Lysis buffer (Raybiotech, USA) and centrifugated at top speed for 20 minutes at 4°C. The assay membranes were incubated in blocking solution for 30 minutes at room temperature. Tissue lysate was added to the membrane and incubated with gentle rocking at 4°C overnight. After washing the membrane,

biotin-conjugated anti-cytokine antibody was added to each membrane for 2 hours at room temperature. The membrane was washed repeatedly, and HRP-conjugated streptavidin secondary antibody reaction was run for 2 hours at room temperature by rocking. Cytokines bound to membranes were evaluated by chemiluminescence assay. Signal quantification was measured by subtracting the background signal using WSE-6100 LuminoGraph (Atto, Japan).

Immunohistochemistry (IHC) analysis

To measure the microglial and astrocyte activation in the rat brain, brain tissues were removed immediately after PET images, fixed in 10% neutral-buffered formalin and made paraffin blocks. The brains were cut into 4 μm-thick sections and IHC analysis was performed as previously described (18). Briefly, the slides were deparaffinized, performed antigen retrieval and blocked endogenous peroxidase activity. After washing in TBS-T buffer, blocking buffer (5% BSA in TBS-T buffer) was added onto the sections of the slides and incubated in a humidified chamber at room temperature for 1 hour. The slides were stained with GFAP (1:100 ratio, GeneTex, USA) or IBA-1 (1:100 ratio, GeneTex, USA) antibodies as primary antibodies at 4°C overnight and used the Dako REAL™ EnVision™ Detection System (Agilent Technologies, Inc., CA, USA). Stained slides were imaged at 10× and 20× through the Vectra 3.0 Automated Quantitative Pathology Imaging System (PerkinElmer). The magnified scanned image files were analyzed using Inform™ 2.2 image analysis software (PerkinElmer).

Statistical Analysis

All data are expressed as mean ± SEM. Medcalc (19.3; MedCalc Software) was used for statistical analysis. For the PET scans and immunofluorescent Iba1 /GFAP cell density values, the differences were compared using two-sample Student's t tests. A p value of < 0.05 was considered significant.

연구결과

The demographics of ^{18}F-THK5351 and ^{18}F-fluorodeprenyl-D_2 in vivo PET/MR imaged experiments are shown in Table 1.

Expression of proinflammatory cytokines and glial activations in LPS induced neuroinflammatory model.

To investigate the proinflammatory reaction in the LPS induced rat, we measured the expression levels of proinflammatory cytokines in the brain homogenates at 24 h post LPS (i.p. 5mg/kg and i.c. 1μg and 10μg). The expression levels of TNF-α, CCL2, and CCL20 in the brain homogenates of the LPS induced rats were higher than those in the control rats, demonstrating that LPS can lead to the occurrence of inflammation and the release of proinflammatory cytokines.

Further evidence that intraperitoneal and intracranial LPS injection produces neuroinflammation was also supported by the observation that microglial and astrocytes density increased in the brain of rats following LPS injection compared to controls. As shown in Fig. 1, the numbers of Iba-1 positive cells intensity for indicating microglial activation were higher in the intraperitoneal LPS injection group than control (4.6 % vs. 3.2 %, $p = 0.045$). The number of GFAP positive cell intensity, a marker of reactive astrocytosis, were also higher in the LPS injection group when compared to control group (3.6 % vs. 2.0 %, $p = 0.028$). In intracranial LPS injection group, the numbers of Iba-1 positive cells intensity were higher than in control group (3.7 % and 5 % (for i.c. LPS 1 ug and 10 ug, respectively) vs. 2.7 %, $p < 0.05$). The number of GFAP positive cell intensity were also higher in the LPS injection group when compared to control group (10.7 and 12.5 % (for i.c. LPS 1 ug and 10 ug, respectively) vs. 3.0%, $p < 0.05$).

In vivo PET image of control group

The time activity curves expressed as SUV and SUVR (reference: cerebellum) of ^{18}F-THK5351 and ^{18}F-fluorodeprenyl-D_2 uptake in all brain regions of control group are shown in Fig. 2. In ^{18}F-THK5351 PET images, the tracer crossed the blood-brain barrier and bound rapidly, with an average time to peak of 60s, the highest radioactivity concentration (expressed as SUV) was seen in the thalamus (2.3 ± 0.2). ^{18}F-THK5351 showed fast washout

Table 1. Demographics of in vivo ^{18}F-THK5351 and ^{18}F-fluorodeprenyl-D_2 small animal PET studies

Study group	^{18}F-THK5351				^{18}F-fluorodeprenyl-D_2			
	n	Age (weeks)	Weight (g)	Inj.dose (mCi)	n	Age (weeks)	Weight (g)	Inj.dose (mCi)
Control	4	9	279.2 ± 30.8	0.50 ± 0.03	4	9	254.1 ± 31.1	0.38 ± 0.03
IP	4	11	317.8 ± 15.9	0.53 ± 0.16	3	11	290.4 ± 42.7	0.50 ± 0.14
IC 1 ug	4	9	344.0 ± 7.8	0.60 ± 0.02	4	9	253.9 ± 7.1	0.56 ± 0.09
IC 10 ug	4	9	254.2 ± 3.1	0.58 ± 0.12	4	9	255.9 ± 6.4	0.46 ± 0.08

The rat weights and injected dose are expressed as means ± SEM for each study group
*Inj.*injected, *IP* intraperitoneal injection group, *IC 1ug*, intracranial injection with LPS 1ug group, *IC 10ug* intracranial injection with LPS 1ug group

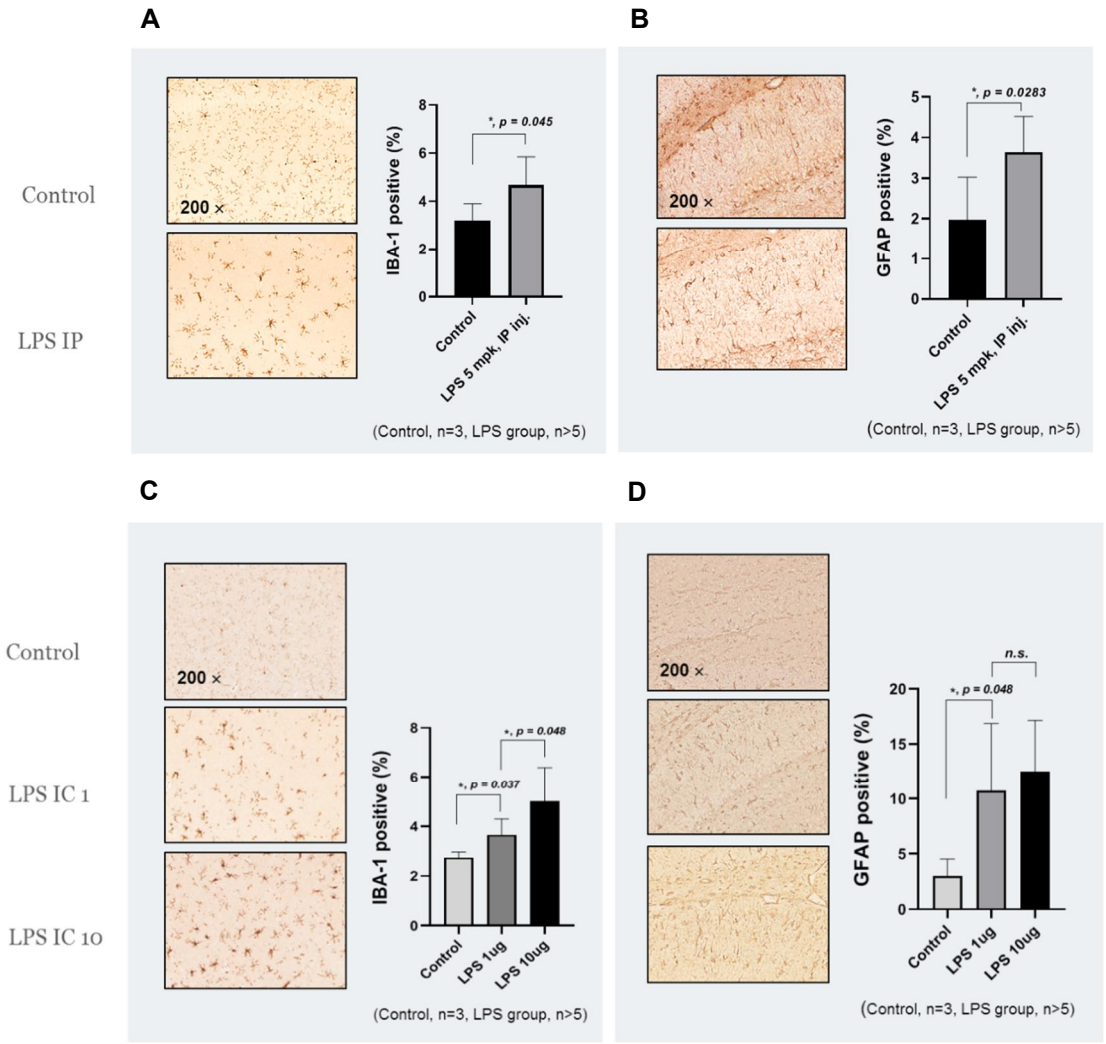

Figure 1. Intense Iba1 immunofluorescent staining (increased size and number of activated microglia) and GFAP immunofluorescent staining (increased size and number of reactive astrocytes) in rats injected with LPS intraperitoneally (A, B) and intracranially (C, D).

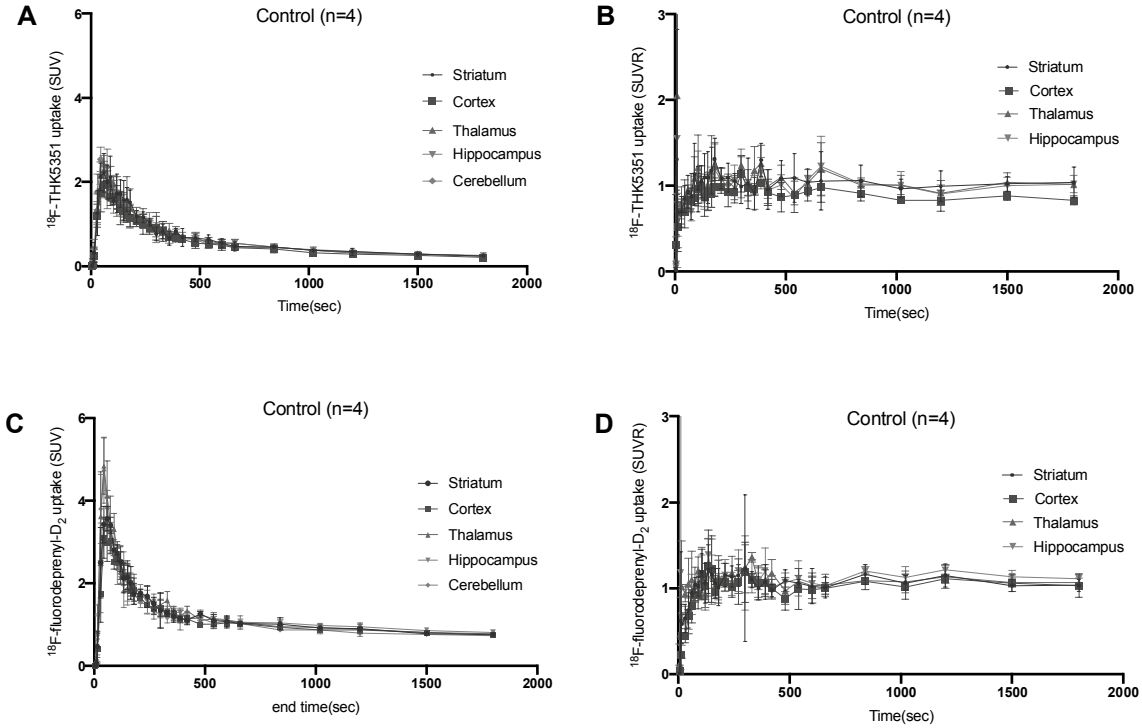

Figure 2. Average time-activity curve expressed as SUV and SUVR for ^{18}F-THK5351 (A, B) and for ^{18}F-fluorodeprenyl-D_2 (C, D) in control group.

with peak to 10 min activity ratio of 4.4 and peak to 30 min activity ratio of 10. On 10-30 min summed image, ^{18}F-THK5351 uptake of cortex was lower than that of cerebellum (0.6 ± 0.0 vs. 0.7 ± 0.0, p=0.02).

^{18}F-fluorodeprenyl-D_2 crossed the blood-brain barrier and bound rapidly either, with an average time to peak of 51s, the highest radioactivity was in the thalamus (4.8 ± 0.7). ^{18}F-fluorodeprenyl-D_2 showed fast washout from cortical, subcortical region and cerebellum with peak to 10 min activity ratio of 3.9 and peak to 30 min activity ratio of 5.2. On 10-30 min summed image, ^{18}F-fluorodeprenyl-D_2 uptake of hippocampal region showed highest uptake and was higher than that of cerebellum (1.9 ± 0.1 vs. 1.7 ± 0.1, p=0.02).

PET images in neuroinflammatory model

Intraperitoneal LPS injection group

The time activity curves of radiotracer uptakes in the evaluated brain regions and representative images of ^{18}F-THK5351 and ^{18}F-fluorodeprenyl-D_2 PET/MR images are displayed in Fig. 3. As shown in Table 2, the uptakes of ^{18}F-THK5351 were significantly higher in whole brain region: striatum, cortex, thalamus and hippocampus and cerebellum in LPS intraperitoneal injection group than those in control group. In ^{18}F-fluorodeprenyl-D_2 PET images, slightly increased uptakes were noted in cortex, hippocampus and cerebellum, but not in other regions.

Intracranial LPS injection group

As shown in Fig. 4. , increased ^{18}F-THK5351 binding was observed in the core VOI, the injection site of 1ug of LPS, with the fold increase ranged from 1.16 to 1.23 (core/contralateral uptake ratio), but not significant in the 10ug of LPS injection group (core/contralateral ratio = 1.13 ± 0.11). No significantly increased ^{18}F-fluorodeprenyl-D_2 uptake was observed in the group injected with either 1ug or 10 ug of LPS (core/contralateral ratio = 1.01 ± 0.04 and 1.09 ± 0.04). For comparison with the control group, all LPS-injected animals were pooled for each tracer. Averages of contralateral uptake in all LPS intracranial injected animals (0.71 ± 0.07 and 1.81 ± 0.10 for ^{18}F-THK5351 and ^{18}F-fluorodeprenyl-D_2, respectively) were not significantly different from uptakes in control

group (0.70 ± 0.8 and 1.81 ± 0.10 for ^{18}F-THK5351 and ^{18}F-fluorodeprenyl-D_2, respectively), supporting the use of the contralateral VOI as reference region.

Table 2. Uptakes of ^{18}F-THK5351 and ^{18}F-fluorodeprenyl-D_2 in intraperitoneal LPS injection group

	^{18}F-THK5351				^{18}F-fluorodeprenyl-D_2			
	SUV		SUVR		SUV		SUVR	
	LPS IP	Control	LPS IP	Control	LPS IP	Control	LPS IP	Control
Striatum	1.19 ± 0.13 **	0.70 ± 0.08	1.01 ± 0.05	0.98 ± 0.05	1.96 ± 0.19	1.81 ± 0.11	1.02 ± 0.08	1.07 ± 0.03
Cortex	1.06 ± 0.08 ***	0.60 ± 0.05	0.89 ± 0.01	0.85 ± 0.05	1.96 ± 0.14 *	1.80 ± 0.06	1.02 ± 0.05	1.06 ± 0.08
Thalamus	1.24 ± 0.13 ***	0.70 ± 0.07	1.05 ± 0.05	0.99 ± 0.04	1.96 ± 0.06	1.81 ± 0.17	1.02 ± 0.02	1.07 ± 0.03
Hippocampus	1.19 ± 0.12 **	0.70 ± 0.09	1.00 ± 0.04	0.98 ± 0.05	2.07 ± 0.10 *	1.91 ± 0.06	1.08 ± 0.03	1.13 ± 0.05
Cerebellum	1.18 ± 0.08 ***	0.71 ± 0.06			1.93 ± 0.04 *	1.70 ± 0.12		

SUV and SUVR are expressed as means ± SD for each study group

LPS IP intraperitoneal LPS injection group

* p <0.05, ** p <0.005, *** p <0.001 significantly higher than control

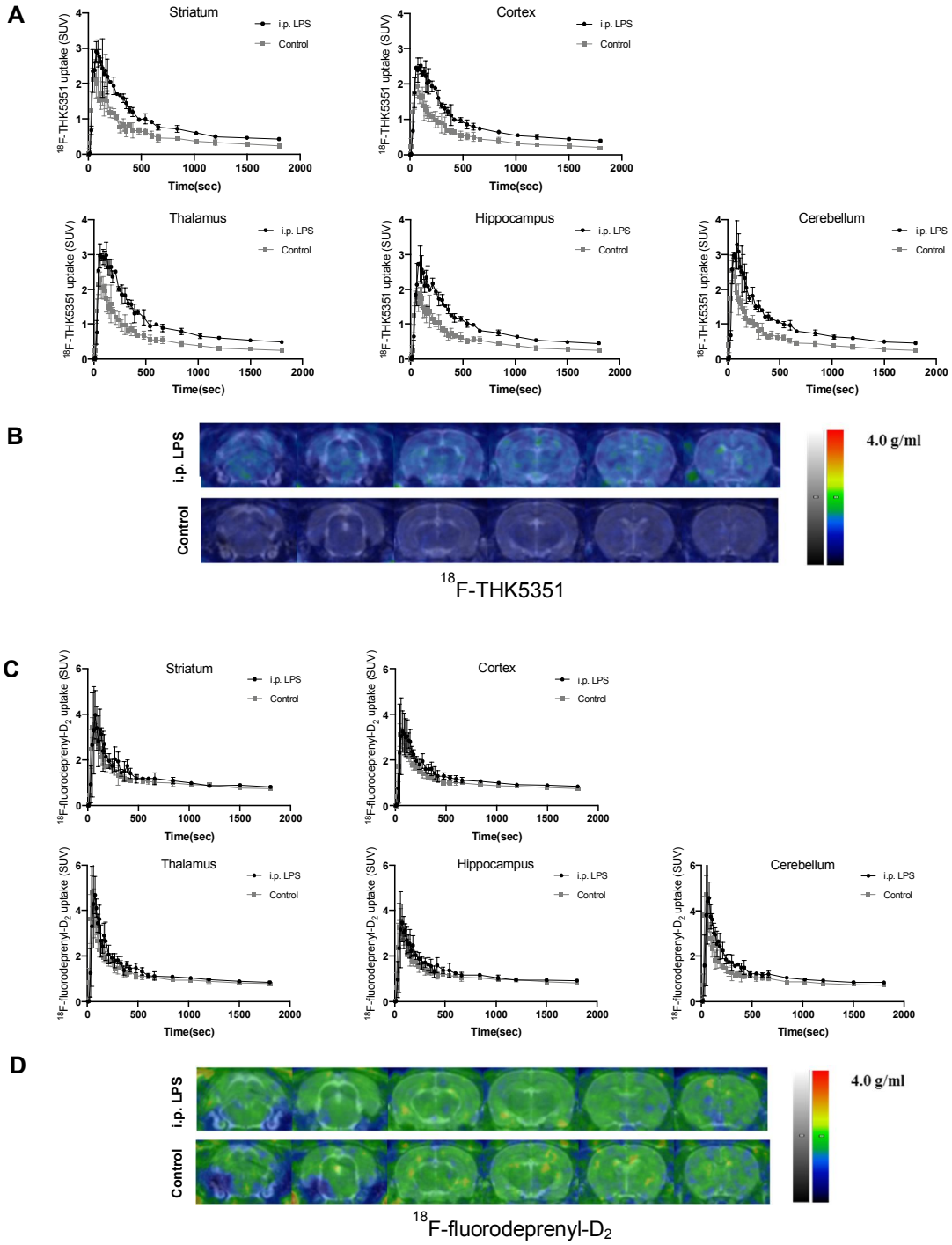

Figure 3. Average time-activity curve and representative PET/MR images for ^{18}F-THK5351 and ^{18}F-fluorodeprenyl-D_2 in rats injected with LPS intraperitoneally.

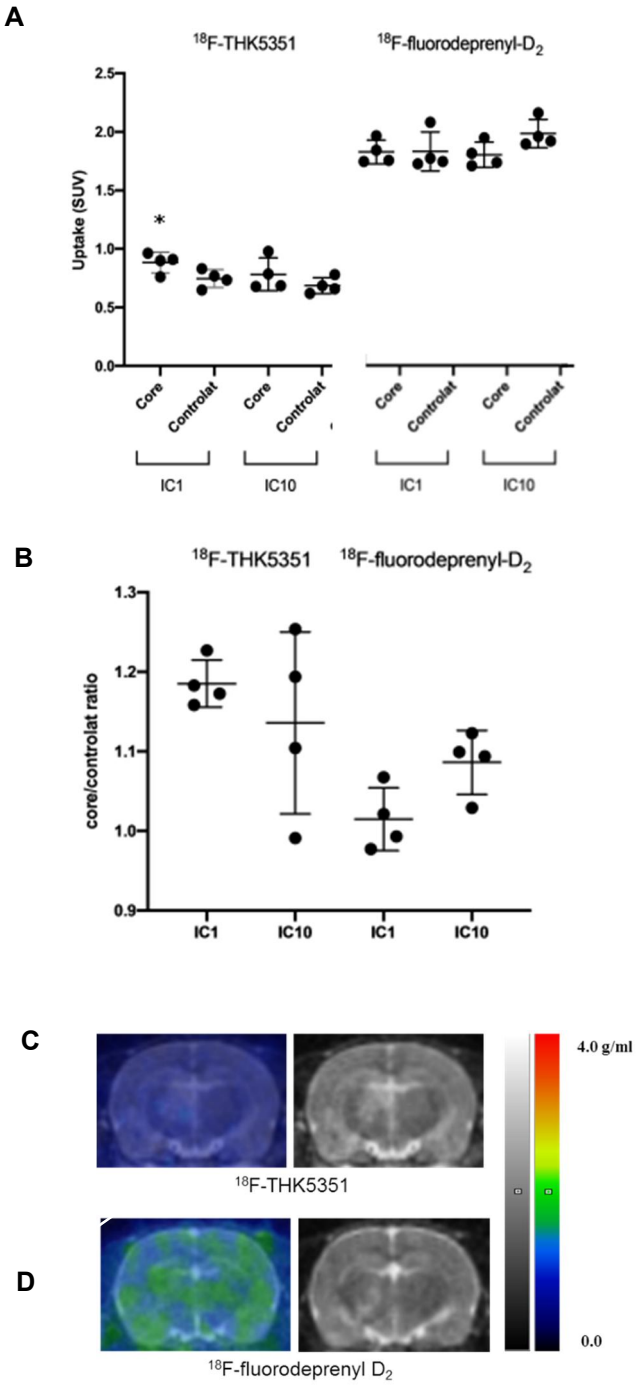

Figure 4. ^{18}F-THK5351 and ^{18}F-fluorodeprenyl-D_2 mean uptake value at 10-30 min summed image (expressed as SUV, mean ± SEM (A) and core/contralateral ratio (B)) of rats injected with LPS intracranially. * Significantly different from the contralateral side of the same tracer (p <0.05). Representative coronal ^{18}F-THK5351 (C) and ^{18}F-fluorodeprenyl-D_2 (D) summed PET/MR images of 10-30 min with T2WI MR image.

고찰

Preclinical studies are valuable for assessment and characterization of tracer behavior, especially where models can be developed to mimic clinically relevant levels of inflammation although results from rodents are not directly transferable to humans due to inter-species differences. The aim of this study was to test the hypothesis that the LPS injected rat brain shows astrocyte activation followed by upregulation of MAO-B activity which would potentially allow the use of ^{18}F-THK5351 and ^{18}F-fluorodeprenyl-D_2 in this animal model as biomarker for the acute neuroinflammation. To date, there is neither reported investigations comparing these two tracers as MAO-B binding radioligands nor looking at their performance in a rat model with neuroinflammation.

In order to validate tracer uptake in different type of neuroinflammatory process and find the feasible neuroinflammatory model for animal PET imaging study, we induced two acute neuroinflammatory model using LPS injection in different manner.

Systemic administration of LPS is an accepted animal model of sepsis. A severe systemic inflammatory condition results in both short- and long-term neurocognitive dysfunction. The administration of LPS contributes to increased neuroinflammation along with damage to the Blood Brain Barrier (BBB), thereby causing amyloid genesis and memory deficiency. Furthermore, LPS-induced brain inflammation is accompanied by neuronal loss and microglia activation, which induce the release of neurotoxic factors, such as inflammatory cytokines (TNF-α, IL-1β, PGE_2, etc.) (19) and widely used in study for neurodegenerative and neuroinflammatory disease including AD. This secondary neuroinflammation induced lower and diffuse neuroinflammation is more variable and difficult to detect than strong, robust neuroinflammatory model induced by neurotoxin direct injection. In our study, after 24h intraperitoneal LPS injection, markedly increased ^{18}F-THK5351 retention was noted in whole brain region without regional differences in changes. ^{18}F-THK5351 might be superior to ^{18}F-fluorodeprenyl-D_2 in detecting astrocyte activation with more favorable signal to noise ratio, especially in diffuse and mild neuroinflammation. However, tracer uptake could be influenced by other factors such as perfusion changes, BBB disruption, and off target binding in tau in this result. In a postmortem Alzheimer's disease study, regional in vivo ^{18}F-THK5351 retention in the neocortex correlated not only with PHF-tau, but also with MAO-B levels in the brain (12). Autoradiography data using ^{11}C-*L*-deprenyl in AD brain tissue, ^{3}H-*L*-deprenyl partly co-

located with tau as measured by ^{3}H-THK5117 (20). A recent study reported that a single LPS intraperitoneal injection increased the levels of phosphorylated tau in the brain of rats (21). Increased ^{18}F-THK5351 retention in whole brain regions in our data may contributed by THK5351 binding to both MAO-B and PHT-tau following LPS induced systemic inflammation. Further studies are required to differentiate between tau protein accumulation and MAO-B uptake and clarify what percentage of the ^{18}F-THK5351 PET signal was derived from MAO-B and PHF-tau.

The neuroinflammatory model induced by intracranial LPS injection was expected to have advantages in evaluation of the spatial extent of inflammation in the brain and be more suitable for validation of imaging tracers in vivo. LPS, on binding to LPS binding protein and CD14, quickly activates the immune system via toll-like receptor 4 (22) and the NFkB pathway to induce an M1 phenotype (23). This model has also the advantage that the animals can be imaged at a much earlier time point and it does not induce overt clinical signs. Another benefit of the unilateral intracranial injection model is that it caused unilateral inflammatory response permitting and internal control. In our study, ^{18}F-THK5351 uptake of direct intracranial LPS injection site increased over contralateral side level with the fold increase ranged from 1.16 to 1.23. However, contrary to our expectation, tracer uptake near LPS injected site was not detectable when visually assessed in the most of experiments. Decreased perfusion, procedure-induced hemorrhage, edema or apoptosis of neural cell might decrease tracer uptake in intracranial injection model. Also, the potential for significant species differences as well recognized for the isozymes of MAO is another considerable factor that explains low binding of ^{18}F-fluorodeprenyl-D_2 in our acute neuroinflammatory rat model.

Limitation of our study is that direct estimation of specific binding was not possible since there is no suitable reference region available for MAO-B binding in this model with diffuse neuropathology, and arterial blood sampling is not logistically feasible. In such cases, it is not unusual to perform qualitative comparison of the regional distribution of the tracer (SUV) in the different groups of animals if the studies are run in parallel. We also performed comparison in ‘non-paired’ animals individually scanned with different tracer. The inherent variability within an experiment of populations makes quantification and comparison more challenging when measuring level of neuroinflammation.

결론

In conclusion, ^{18}F-THK5351 showed increased retention in rat brain with acute neuroinflammation. But ^{18}F-fluorodeprenyl-D_2 did not show significantly increased retention. These results suggest that ^{18}F-fluorodeprenyl-D_2 might not be suitable for detection of acute neuroinflammation in rat brain. Further studies are needed to determine the specificity of ^{18}F-THK5351.

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국문 요약

연구배경 및 목적: 신경염증은 아교세포, 반응성 성상세포의 활성을 동반하며 이는 여러 신경질환에서 주요 병리기전으로 알려짐에 따라 그 중요성이 대두되고 있다. 비 침습적으로 신경 염증을 영상화 할 수 있는 진단능은 신경염증의 병리 기전과 치료 효과를 모니터링 하는 데 필수 적이다. 모노아민산화효소(MAO-B)는 성상 세포에 대한 표적 중 하나로 신경 염증의 PET 영상화를 위한 바이오 마커로 기대되고 있고 이를 표적으로 방사성 리간드들이 개발되고 있으며 급성 신경 염증 모델에서의 MAO-B 활성 검출능에 대한 연구가 필요하다. 이에 대해 본 연구에서는 급성 신경 염증 과정에 관여하는 MAO-B 활성도를 방사성 추적자인 ^{18}F-fluorodeprenyl-D_2 과 ^{18}F-THK5351를 이용해 영상화 하고 정량화 하고자 하였다.

대상 및 방법: SD 랫드를 복강 내 독소 주입 군, 두개 내 독소 주입 군의 두 군으로 나누어 복강내 주입 군은 5-10 mg/kg의 lipopolysaccharide (LPS)를 복강내 주사하였고 두개내 주입 군은 두 그룹으로 나누어 각 1ug, 10ug의 LPS를 두개 내로 직접 주사하였다. LPS 주사 16-24 시간 후 ^{18}F-THK5351 (n=4, 각 군, 그룹 별) 또는 ^{18}F-fluorodeprenyl-D_2. (n=4, 각 군, 그룹 별)을 주사한 후 PET/MRI를 이용하여 30분 동안의 동적 영상을 획득하였다. 표준섭취화계수 (standardized uptake value: SUV), 섭취비로 표현한 시간-방사능 곡선을 구하였고 섭취를 정량화 하기 위해 10-30분 영상에서 관심영역내의 섭취 정도를 대조군, 주사 부위 반대쪽 반구의 섭취와 비교 분석하였다. 사이토카인 분석 및 면역조직 화학 분석을 시행하여 이를 체내영상검사 결과와 비교하였다.

결과: 정상 대조군 개체에서 ^{18}F-THK5351와 ^{18}F-fluorodeprenyl-D_2 모두 혈액-뇌 장벽을 통과하고 빠르게 섭취된 후 빠져나가는 양상을 나타내었다. 복강 내 LPS 섭취 군에서 ^{18}F-THK5351 섭취는 피질, 피질하영역, 소뇌 영역에서 모두 대조군에 비해 증가하였으며 (1.17 ± 0.07 vs. 0.68 ± 0.05, p <0.001) ^{18}F-fluorodeprenyl-D_2 섭취도 대조군에 비해 증가한 양상이었으나 통계적 유의성은 없었다 (1.97 ± 0.06 vs. 1.81 ± 0.08). 두개 내 LPS 주사 군에서, ^{18}F-THK5351는 LPS

주사부위에서 증가된 섭취를 보였으나 ^{18}F-fluorodeprenyl-D_2 섭취는 반대측 섭취에 비해 유의한 증가를 보이지 않았다 (core/contralateral ratio = 1.16 ± 0.07 vs. 1.05 ± 0.08).

결론: ^{18}F-THK5351는 급성 신경 염증이 있는 랫드 뇌에서 증가된 섭취를 나타내었다. ^{18}F-fluorodeprenyl-D_2 은 유의한 섭취 증가를 보이지 않았으며 이는 ^{18}F-fluorodeprenyl-D_2 가 랫드 뇌에서 급성 신경 염증의 진단에 적합하지 않을 수 있음을 시사한다. ^{18}F-THK5351 섭취의 특이도를 확인하기 위한 추가 연구가 필요하다.