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研究課題名（和文）Positron emission tomography imaging of neuroinflammation

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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：急性および慢性神経炎症モデルマウスを用いて、炎症プロセスに関与する分子であるMAGL、P2X7R、CSF1RのPETイメージングを実施した。その結果、炎症部位においてP2X7RとCSF1Rの放射性リガンドの集積が増加していることがわかり、P2X7R-PETとCSF1R-PETが多様な神経炎症疾患における反応性ミクログリアのイメージングに有効であることが示唆された。さらに、MAGL-PETをMAGL阻害剤の投与量と投与方法を決定するため、阻害薬投与後のMAGLへの占有率の時間変化の推定に応用した。最適な治療計画のもと、タウオパシーのマウスモデルにおけるMAGL阻害の治療可能性を検討した。

研究成果の学術的意義や社会的意義
This work has validated CSF1R and P2X7R as PET imaging biomarkers for microgliosis, allowing us to not only study the pathophysiological roles of those receptors in brain disorders, but also to explore neuroinflammatory processes from different angles.

研究成果の概要（英文）：PET imaging of MAGL, P2X7R, and CSF1R, molecules involving in microgliosis and inflammatory processes has been carried out in mouse models of acute and chronic neuroinflammation. We have found an increased P2X7R and CSF1R radioligands retention in inflammatory brain regions. Furthermore, the pharmacokinetics of 11C-GW2580, a novel tracer for CSF1R has been quantified and compared with that of a reported CSF1R radioligand in mice and monkeys. The study demonstrated that 11C-GW2580 enables detection of microglial activation with higher sensitivity than the reported CSF1R tracer. Our findings suggest the feasibility of P2X7R- and CSF1R-PET for imaging of reactive microglia in diverse neuroinflammatory diseases. MAGL-PET was applied to determine the dose and regimen of an MAGL inhibitor by estimating the time-course occupancy of MAGL after drug administration. With the optimal treatment plan, the therapeutic potential of MAGL inhibition in a mouse model of tauopathy was investigated.

研究分野：Molecular imaging

キーワード：PET　CSF1R　P2X7R　MAGL　Neuroinflammation

１．研究開始当初の背景

Neuroinflammation is a common feature in many brain disorders, such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, and schizophrenia. Albeit great efforts have been put into characterizing neuroinflammatory responses under various conditions, little is known about the underlying mechanisms and the relations between inflammation and other pathological processes, including neurotoxic protein deposition, oxidative stress, and loss of synapses and neurons, in the disease brain. Lacking biochemical markers of inflammation and limited sensitivity and specificity of these markers are major barriers to untangling complex pathways involving in neuroinflammation.

２．研究の目的

In this study, we seek to investigate new *in-vivo* imaging markers, with the aid of positron-emission tomography (PET) and proper PET probes, in the dynamics of CNS inflammation. We focus on three endogenous molecules, monoacylglycerol lipase (MAGL), colony stimulating factor 1 receptor (CSF1R), and P2X purinoceptor 7 (P2X7R) which have been suggested to play important roles in neuroinflammatory cascades, in animal models of acute and chronic CNS inflammation.

３．研究の方法

CSF1R: A novel CSF1R radioligand ^{11}C-GW2580 was developed via C-11 labeling of a CSF1R inhibitor, GW2580. Dynamic PET imaging of CSF1R with ^{11}C-GW2580 and a reported CSF1R radiotracer, ^{11}C-CPPC, was conducted in mice with lipopolysaccharide (LPS)-induced acute inflammation and *App*$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$-knock-in (APP-KI) mice modeling chronic neuroinflammation. In parallel with CSF1R imaging, PET imaging of translocator protein 18 kDa (TSPO) was performed to visualize inflammatory gliosis. In addition, imaging of CSF1R with these two radioligands was carried out in a rhesus monkey at baseline and under homologous blockade.

P2X7R: PET imaging of P2X7R with ^{18}F-JNJ64413739 was carried out in rTg4510 mice, a mouse model of tauopathy, and control mice at baseline and blocking conditions to characterize the pharmacokinetics and specificity of the radioligand. Additionally, time-course changes in the level of P2X7R, TSPO expression, tau burden, and white matter integrity were assessed with ^{18}F-JNJ64413739-, ^{11}C-AC5216-, and ^{18}F-PBB3-PET, and diffusion tensor imaging (DTI), respectively, in those animals.

MAGL: The distribution and relative concentration of MAGL were assessed by ^{18}F-T401-PET in LPS-injected and rTg4510 mice. Furthermore, MAGL occupancy by an MAGL inhibitor was estimated by a target occupancy experiment carried out with ^{18}F-T401-PET in FVB mice. A proper dose regimen was subsequently determined. With the treatment plan, multi-model in vivo imaging was carried out longitudinally in rTg4510 mice with vehicle or MAGL inhibitor treatment to examine the extent of neurodegeneration, tau burden, TSPO expression, and MAGL concentration in their brains.

４．研究成果

CSF1R: Both ^{11}C-GW2580 and ^{11}C-CPPC exhibited increased uptake in the lesioned striata of LPS-injected mice and in the forebrains of APP-KI mice, spatially in agreement with an increased TSPO radioligand retention. Moreover, ^{11}C-GW2580 captured changes in CSF1R availability more sensitively than ^{11}C-CPPC, with a larger dynamic range and a smaller inter-individual variability, in these model animals (**Fig. 1**). Finally, treatment of a monkey with a corresponding non-radiolabeled compound reduced the uptake of ^{11}C-GW2580 in the brain by 30% but did not induce homologous blockade of ^{11}C-CPPC retentions (**Fig. 2**). Those findings demonstrated that ^{11}C-GW2580-PET captured increased CSF1R density in mice brains modeling acute and chronic neuroinflammation with higher sensitivity than a reported radioligand, and displayed saturable binding in the monkey brain, potentially providing an imaging-based quantitative biomarker for reactive microgliosis.

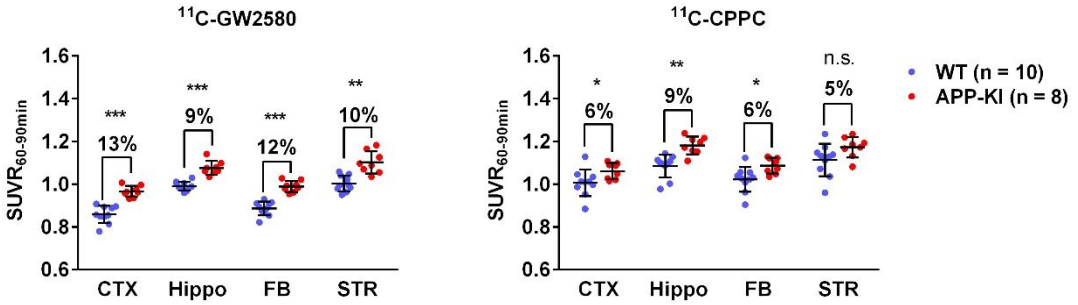

Fig. 1. Comparison of ^{11}C-GW2580 or ^{11}C-CPPC uptakes in the neocortex (CTX), hippocampus (Hippo), entire forebrain (FB), and striatum (STR) between wild-type control and APP-KI mice. ^{11}C-GW2580 yielded greater inter-group difference and smaller inter-subject variability than ^{11}C-CPPC. *p<0.05, **p<0.01, ***p<0.001, n.s., not significant. Error bars indicate standard deviation. SUVR, standardized uptake value ratio.

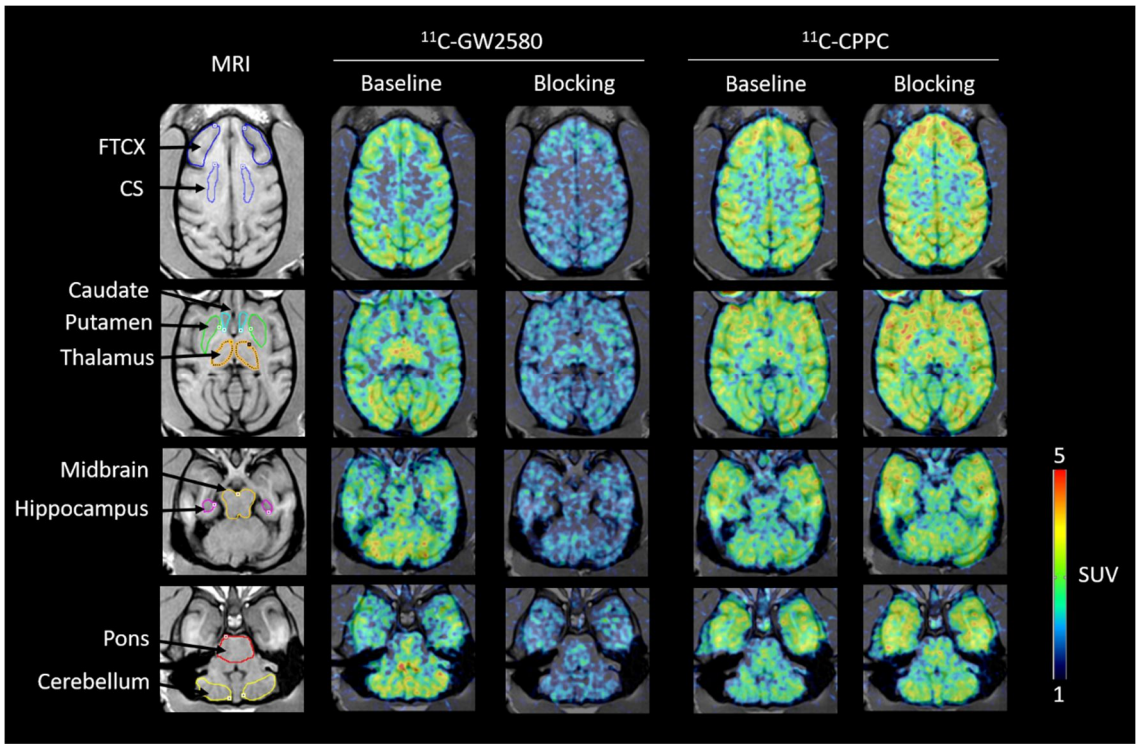

Fig. 2. Representative transverse planes of ^{11}C-GW2580 and ^{11}C-CPPC $SUV_{60\text{-}120min}$ images of a monkey brain superimposed on the monkey's own magnetic resonance images at baseline and with a homologous blocker treatment.

P2X7R: The *in-vivo* PET imaging of P2X7R displayed an increased radioligand retention in the forebrains of aged rTg4510 mice relative to non-Tg control mice (**Fig. 3(a)**), whereas no difference was observed between those animals at three month (n=4 each genotype). In addition, we have found an age-dependent increase of the uptake of ^{18}F-JNJ64413739 in tau-rich regions of rTg4510 mice (**Fig. 3(b)**). Such alternation correlated positively with tau accumulation and TSPO expression and negatively with white matter integrity (**Figs. 3(b-e)**). Treatment with both homologous and heterologous blockers slightly reduced the tracer uptake in the brains of non-Tg mice by 1-5% (n=2), whereas lowered the tracer binding in the forebrains of rTg4510 mice (n=2) to the level similar to that in non-Tg mice. These findings indicate that the density of P2X7R was low in the mouse brain under the physiological condition, and the elevated density of P2X7R in response to tau accumulation was captured by ^{18}F-JNJ64413739-PET.

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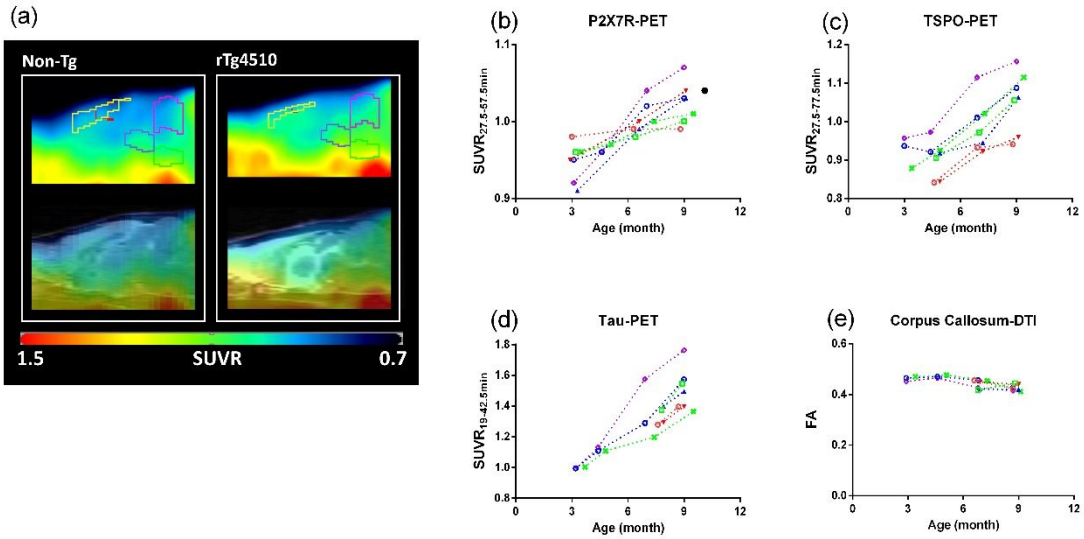

Fig. 3. (a) Representative sagittal $SUV_{15\text{-}90min}$ images of ^{18}F-JNJ64413739 in the brains of non-Tg and rTg4510 mice (top row) superimposed on the templates of magnetic resonance image (bottom row). (b-d) Quantification of PET images with standardized uptake value ratio (SUVR) using cerebellum as the reference region revealed an age-dependent increase of P2X7R and TSPO expression, and tau accumulation in the hippocampus of rTg4510 mice. (e) Those animals also displayed white matter damage at late stage of tauopathy, as determined by reduced fractional anisotropy (FA). DTI, diffusion tensor imaging.

MAGL: PET imaging of MAGL with ^{18}F-T401 showed that the level of MAGL did not alter in both acute and chronic inflammatory conditions. We then redirected our study to investigating the therapeutic potential of MAGL inhibition in neuroiflammatory diseases. PET imaging of MAGL revealed that repetitive administration of JZL184, an MAGL inhibitor to mice with a proper dose and treatment interval led to 50%-100% occupancy of MAGL. With this treatment plan, our ongoing study showed that chronic treatment of rTg4510 mice with JZL184 from 3 month to 6 month greatly reduced neuroinflammation, tau accumulation, and neurodegeneration (**Fig. 4**).

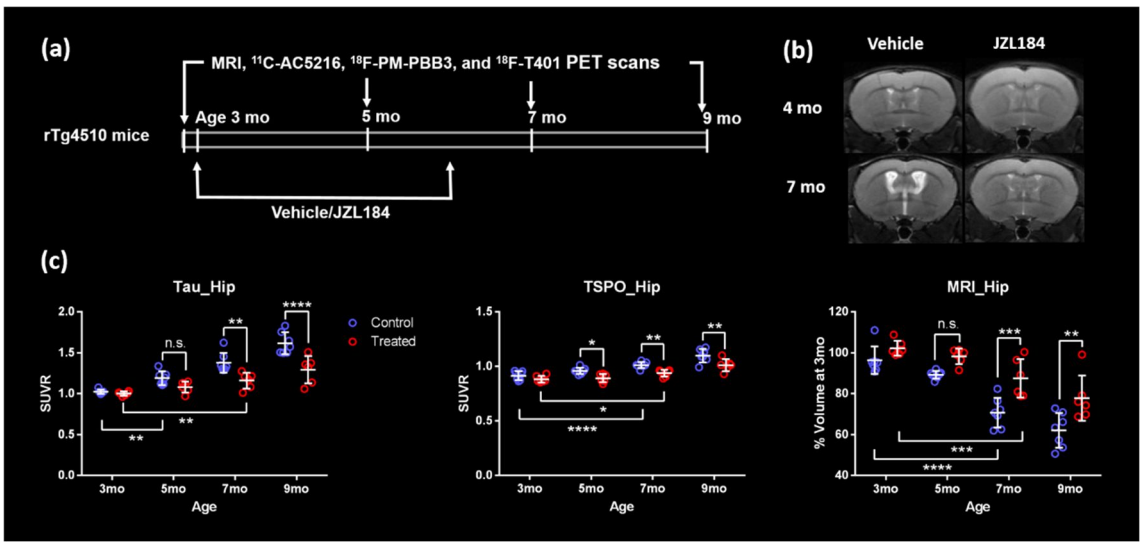

Fig. 4. (a) Study design for longitudinal assessment of MAGL inhibition in inflammation-related pathologies. (b, c) JZL184 treatment reduced neuroinflammation, delayed tau accumulation and rescued neurons in the forebrain regions. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Hip, hippocampus, SUVR, standardized uptake value ratio.

In summary, we have successfully developed and evaluated novel radioligands for the imaging of microgliosis. PET imaging of CSF1R and P2X7R demonstrated the involvement of those receptors in the neuroinflammatory processes induced by the accumulation of toxic amyloid-β

and tau. Additionally, anti-inflammatory treatment via inhibition of the function of MAGL might be benefit to alleviate pathological alternations in tauopathy. Our study provided useful tools to explore the roles played by neuroinflammation in neurodegeneration that will facilitate anti-inflammatory drug development against a variety of neurodegenerative disorders.

５．主な発表論文等

〔雑誌論文〕　計5件（うち査読付論文　5件／うち国際共著　5件／うちオープンアクセス　3件）

１．著者名 Milicevic Sephton Selena、Zhou Xiaoyun、Thompson Stephen、Aigbirhio Franklin I.	４．巻 51
２．論文標題 Preparation of the Serotonin Transporter PET Radiotracer 2-({2-[(Dimethylamino)methyl]phenyl}thio)-5-[18F]fluoroaniline (4-[18F]ADAM): Probing Synthetic and Radiosynthetic Methods	５．発行年 2019年
３．雑誌名 Synthesis	６．最初と最後の頁 4374～4384
掲載論文のDOI（デジタルオブジェクト識別子） 10.1055/s-0039-1690522	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Qiao Luxi、Fisher Emily、McMurray Lindsay、Milicevic Sephton Selena、Hird Matthew、Kuzhuppilly‐Ramakrishnan Nisha、Williamson David J.、Zhou Xiouyun、Werry Eryn、Kassiou Michael、Luthra Saijinder、Trigg William、Aigbirhio Franklin I.	４．巻 14
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３．雑誌名 ChemMedChem	６．最初と最後の頁 982～993
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/cmdc.201900023	査読の有無 有
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３．雑誌名 Journal of Cerebral Blood Flow & Metabolism	６．最初と最後の頁 1-13
掲載論文のDOI（デジタルオブジェクト識別子） 10.1177/0271678X211004146	査読の有無 有
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2．論文標題 Distinct microglial response against Alzheimer's amyloid and tau pathologies characterized by P2Y12 receptor	5．発行年 2021年
3．雑誌名 Brain Communications	6．最初と最後の頁 1-19
掲載論文のDOI（デジタルオブジェクト識別子） 10.1093/braincomms/fcab011	査読の有無 有
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1．著者名 McMurray Lindsay、Macdonald Jennifer A.、Ramakrishnan Nisha Kuzhuppilly、Zhao Yanyan、Williamson David W.、Tietz Ole、Zhou Xiaoyun、Kealey Steven、Fagan Steven G.、Smolek Tomas、Cubinkova Veronika、Zilka Norbert、Spillantini Maria Grazia、Tolkovsky Aviva M.、Goedert Michel、Aigbirhio Franklin I.	4．巻 12
2．論文標題 Synthesis and Assessment of Novel Probes for Imaging Tau Pathology in Transgenic Mouse and Rat Models	5．発行年 2021年
3．雑誌名 ACS Chemical Neuroscience	6．最初と最後の頁 1885～1893
掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acschemneuro.0c00790	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計1件（うち招待講演　0件／うち国際学会　1件）

1．発表者名 Xiaoyun Zhou, Chie Seki, Maiko Ono, Masayuki Fujinaga, Ming-Rong Zhang, Takashi Saito, Takaomi C Saido, Tetsuya Suhara, Bin Ji, Makoto Higuchi
2．発表標題 Head-to-head comparison of two colony stimulating factor-1 receptor PET ligands, [11C]GW2580 and [11C]CPPC, for imaging of acute and chronic neuroinflammatory changes in mice
3．学会等名 Brain & Brain PET 2019（国際学会）
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〔図書〕　計0件

〔産業財産権〕

〔その他〕

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6．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関