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科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Evaluation of new generation translocator protein ligand efficacy in a mouse model of Alzheimer`s disease.

研究課題名（英文）Evaluation of new generation translocator protein ligand efficacy in a mouse model of Alzheimer`s disease.

研究代表者

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研究成果の概要（和文）：以前の研究では、TSPO(トランスロケーター蛋白)リガンドがアルツハイマー病マウスにおいて保護的作用を示した。当研究では三つの次世代TSPOリガンドの潜在的適切性を検証することを目的としている。新しいリガンドの脳透過性及びTSPOへの結合性をマウスの生体脳とヒトの死後脳で調べた。さらに記憶メモリにたいする効果をマウスで調べた。三つのリガンドはいずれもメモリ機能を改善した。そのメモリ改善の作用機序は脳内モルモン産生促進によるものと考えられている。我らの結果はこれらのTSPOリガンドがアルツハイマー病治療に有望な候補物になる可能性を示唆した。

研究成果の概要（英文）：Our previous work has demonstrated that ligands of the translocator protein (TSPO) are protective in a mouse model of Alzheimer`s disease. The purpose of this study was to characterize the potential suitability of three new generation TSPO ligands. We tested the ability of the new generation TSPO ligands to penetrate the brain of living mice, as well as their ability to bind TSPO in human brain sections. Next we assessed their effect on memory, anxiety and depression-related behaviors in mice. All three TSPO ligands improved memory function and one also reduced depression-related behavior. TSPO ligands are thought to improve memory by increasing brain hormone production. Confirming this mechanism of action, memory improvements in TSPO-ligand treated mice were blocked by inhibition of hormone production. Our findings indicate these new TSPO ligands may be promising therapeutic candidates for the treatment of Alzheimer`s disease.

研究分野：　Neuroscience

キーワード：　Alzheimer`s　Dementia　hormone replacement

１．研究開始当初の背景

Ligands of the translocator protein (TSPO) have been identified as promising candidate therapeutic agents for several neurodegenerative disorders due to their ability to enhance neurosteroidogenesis. We have previously demonstrated that the classic TSPO ligands, Ro5-4864 and PK11195, increase brain steroid levels, reduce Aβ accumulation, and improve cognition in a mouse model of AD.

２．研究の目的

The overall goal of this proposal is to identify and characterize new generation ligands of the translocator protein (TSPO) that show strong potential for translational application for the prevention and treatment of Alzheimer's disease (AD). Here we evaluated the steroidogenic efficacy and acute behavioral effects of three TSPO imidazopyridine ligands, CLINDE, PBR175, and PBR162 (Table 1), which we have previously shown to be non-toxic in vitro. CLINDE, which exhibited the most favorable behavioral and pharmacokinetic profile, was investigated further for its specificity and binding affinity in human brain.

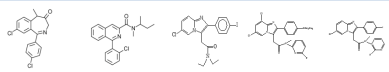
	First Generation		Second Generation		
	Ro5-4864	PK11195	CLINDE	PBR-175	PBR-162
Structure					
Derivative	Benzodiazepine	Isoquinoline	Imidazopyridine acetamide		

Table 1. TSPO ligands for evaluation.

３．研究の方法

The acute behavioural effects of the three novel TSPO ligands were compared with Ro5-4864, in 3 month old castrated C57BL/6J mice. Two hours after ligand injection (3 mg/kg i.p.), anxiety-, depression-, and memory-related behaviors were assessed in the elevated-plus maze, open field maze, tail-suspension test, and object recognition tests respectively. Brain steroids levels were measured by LC-MS/MS. To determine if the behavioral effects of TSPO ligands were mediated via increased neurosteroidogenesis, mice were pretreated with a steroidogenesis inhibitor, aminogluthetimide (10mg/kg). The pharmacokinetic properties of the novel ligands were assessed by competitive PET imaging using [11C]PK-11195. The specificity of action of CLINDE was determined in TSPO knockout mice, and the affinity for TSPO (Ki value) in human brain homogenate was investigated in vitro by competitive binding assay using [11C]PK-11195.

４．研究成果

CLINDE and PBR175 improved learning and memory performance equally well as Ro5-4864 (Fig. 1). These improvements were completely ablated by aminogluthetimide, confirming that the beneficial effects were mediated through an increase in neurosteroidogenesis (Fig. 1). CLINDE also improved depression-related behaviors (Fig. 2). Exploratory and locomotor activity was unaffected by the TSPO ligands.

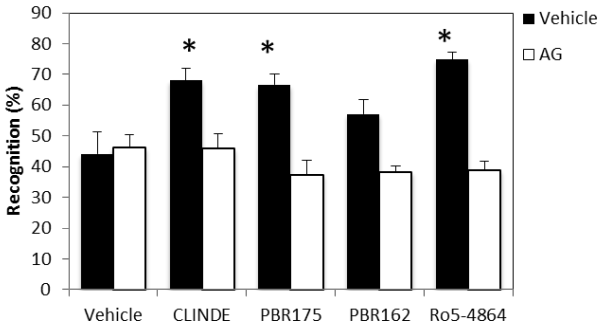


Fig. 1. New generation TSPO ligands rapidly improve learning and memory performance in the object recognition task in mice. Learning and memory improvements were completely ablated through pretreatment with aminoglutethimide (AG), an inhibitor of steroidogogenesis. One-way ANOVA. $p<0.05$ compared to vehicle-treated group.

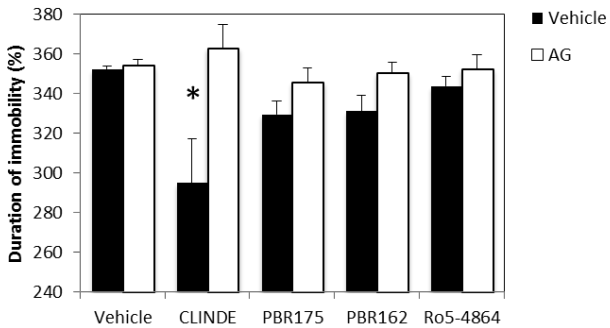


Fig. 2. New generation TSPO ligand, CLINDE, reduces depression-related behavior in the tail suspension test. Inhibition of steroidogenesis through pretreatment with aminoglutethimide (AG) completely blocked the antidepressive effect of CLINDE. One-way ANOVA, $p<0.05$ compared to vehicle treated group.

Competitive PET studies indicated that CLINDE showed the most long lasting binding to TSPO (Fig. 3).

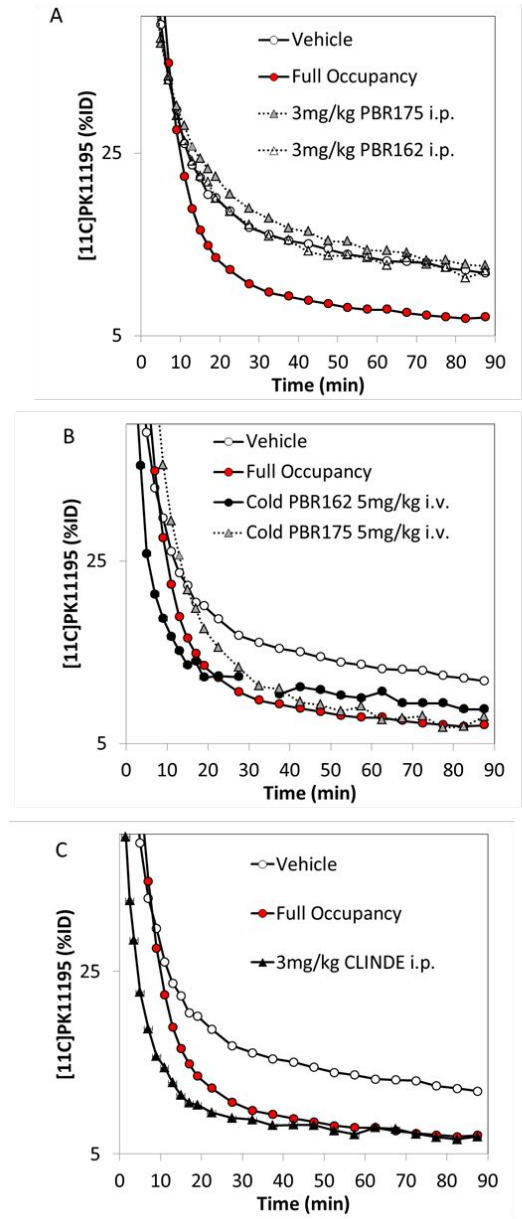


Fig. 3. TSPO occupancy assessed by PET. A, Full brain TSPO occupancy following i.v. injection of PBR-162 and PBR-175, indicating both ligands can cross the blood brain barrier. B, PBR175 and PBR162 undetectable in brain 2hrs post i.p. injection due to rapid metabolism. C, CLINDE (i.p. 2hr prior to scan) rapidly penetrates the brain with high occupancy in the hippocampus (% Occupancy = 93.14 ± 6.86 %).

Since CLINDE exhibited the most favourable behavioral and pharmacokinetic profile, its specificity was confirmed in TSPO knockout (TSPO-KO) mice, with no effect of CLINDE observed on either learning and memory or depression related behavior in the absence of functional TSPO (Fig. 4).

The binding affinity of CLINDE in human high affinity binder (HAB) and low affinity binder (LAB) brain was compared with the prototypic TSPO ligand, PK-11195 (Fig. 5). CLINDE has approximately 5 times higher affinity to HAB than LAB.

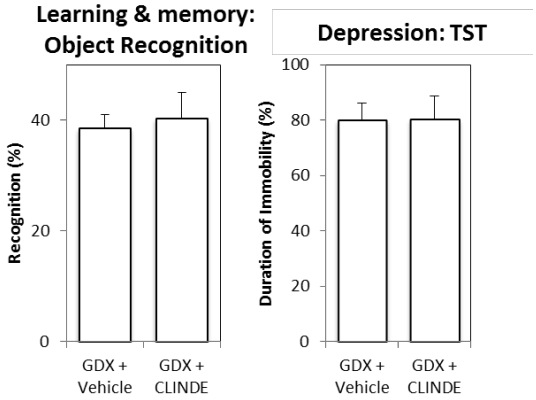
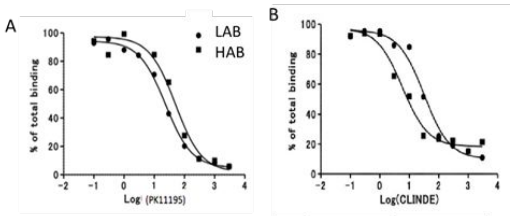


Fig. 4. Behavioral effects of CLINDE specific for TSPO. No effect of CLINDE on learning and memory performance in the object recognition task or depression-related behavior in the tail suspension test (TST) was observed in TSPO-KO mice.



Ligand	Sample	IC50	KI	$R_{(LAB/HAB)}$
PK11195	Non-binder	23.59	21.47	0.5
	Binder	50.46	45.94	
CLINDE	Non-binder	31.85	29.74	5.4
	Binder	5.86	5.47	

Fig. 5. CLINDE binding affinity in human brain. Competition assay with [^{11}C]PK11195 in human brain homogenate from high affinity binder (HAB) and low-affinity binder (LAB) samples the presence of increasing concentrations of unlabeled PK11195 (A) and CLINDE (B). $R_{(LAB/HAB)}$=Ki ratio of LAB to HAB

These findings indicate that CLINDE and PBR175 rapidly promote neurosteroidogenesis, thereby enhancing learning and memory in vivo. These second generation TSPO ligands are promising therapeutic candidates with improved pharmacokinetic properties compared to classic TSPO ligands.

5．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　2　件）

[1] Porcu, P., Barron, A.M., Frye, C., Yang, S-Y., Melcangi, R., Panzica, G. Neurosteroidogenesis today: Novel targets for neurosteroid synthesis and action and their

relevance for translational research. Journal of Neuroendocrinology. 28(2). (2016).

[2] Verdile, G.V., Asih, P. Barron, A.M., Whajoepramono, E.J., Ittner, L.M., Martins, R.N. The Impact of Luteinizing hormone and Testosterone on beta amyloid (Aβ) accumulation: animal and human clinical studies. Hormones and Behavior. 76: 81-90. (2015).

〔学会発表〕（計　3　件）

[1] Barron, A.M., Asih, P.R., Ji, B., Katsifis, A., Pike, C.J., Martins, R.N., Higuchi, M. Translocator protein as a therapeutic target for Alzheimer`s disease. International Steroids and Nervous System Meeting. Torino, Italy. 14-18 February 2015.

[2] Asih, P.R., Bin, J., Katsifis, A., Mattner, F., Caruso, D., Melcangi, R.C., Higuchi, M., Martins, R.N., Barron, A.M. *In* vivo evaluation of the therapeutic potential of novel translocator protein (TSPO) ligands for the treatment of Alzheimer's disease. Alzheimer`s Association International Conference. Washington, USA. 18 – 23 July 2015. Alzheimer`s & Dementia. 11 (7): P156.

[3] Asih, P.R., Barron, A.M., Bin, J., Katsifis, A., Mattner, F., Verdile, G., Gupta, V., Trengrove, R., Martins, R.N. Novel translocator protein (TSPO) ligands for the potential treatment of Alzheimer's disease: A next generation alternative to conventional hormone therapy. Boston, USA. 12 – 17 July 2014. Alzheimer`s & Dementia. 10 (4): P860. (2014).

〔図書〕（計　0　件）

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○出願状況（計　0　件）

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発明者：
権利者：
種類：
番号：
出願年月日：
国内外の別：

○取得状況（計　　件）

名称：
発明者：
権利者：
種類：
番号：
取得年月日：
国内外の別：

〔その他〕
ホームページ等

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