
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.
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.

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, aminoglutethimide (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. 研究結果
CLINDE and PBR175 improved learning and memory performance equally well as Ro5-4864 (Fig. 1). These improvements were completely ablated by aminoglutethimide, 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.](image1)

![Fig. 2. New generation TSPO ligand, CLINDE, reduces depression-related behavior in the tail suspension test.](image2)

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

**Fig. 5.** CLINDE binding affinity in human brain. Competition assay with [¹⁴C]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). \( K_{D}/K_{i} \) 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. 主要発表論文等
（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）


〔学会発表〕（計 3 件）
