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Research report

# TSPO ligand PK11195 alleviates neuroinflammation and beta-amyloid generation induced by systemic LPS administration

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ABSTRACT

Translocator protein 18 kDa (TSPO) is now an attractive drug target for controlling neuroinflammation. Studies applying TSPO ligands to neurodegenerative diseases, especially Alzheimer's disease (AD), were rare. Our study was aimed to evaluate the effect of PK11195, a specific TSPO ligand, in an animal model of neuroinflammation caused by systemic LPS administration. C57/BL6 mice were treated with lipopolysaccharide (LPS, 500 μg/kg, i.p.) three days after PK11195 administration (3 mg/kg, i.p.). The drugs were not discontinued until the mice were sacrificed. Cognitive function was assessed by Morris water maze (MWM) seven days after LPS injection. Chronic LPS-injection in mice was characterized by cognitive dysfunction, increased expression of cyclooxygenase (COX)-2 and TSPO, elevated Aβ content with increased expression of β-site APP cleaving enzyme-1 (BACE-1) and insulin-degrading enzyme (IDE) as well as decreased brain progesterone and brain-derived neurophic factor (BDNF) level. PK11195 pretreatment protected cognitive function in LPS-injected animals and normalized the inflammatory proteins. Moreover, PK11195 pre-administration decreased elevated hippocampal $A\beta_{x-42}$ levels and increased brain levels of progesterone, allopregnanolone. However, LPS-induced BDNF decrease was not reversed by PK11195 administration. Our data demonstrated that PK11195 could protect cognitive deficits induced by chronic LPS administration. The underling mechanism may involve alleviated neuroinflammation, increased synthesis of neurosteroid and decreased Aβ accumulation accompanied by down-regulation of BACE-1.



## 1. Introduction

Translocator protein 18 kDa (TSPO) is a protein primarily localized in the outer membrane of mitochondria in peripheral organs and brain. Translocation of cholesterol from the cytoplasm into the mitochondria for steroidogenesis is one of the important functions of TSPO (Papadopoulos et al., 2006). TSPO also plays an important role in modulating inflammation. TSPO expression is markedly increased not only in brain injury but also in many kinds of neurodegenerative and neurological diseases, including Alzheimer's disease (AD) (Edison et al., 2008; Yasuno et al., 2008), frontotemporal dementia (Cagnin et al., 2004), multiple sclerosis (Harberts et al., 2013; Rissanen et al., 2014), Huntington's disease (Pavese et al., 2006), amyotrophic lateral sclerosis (Zurcher et al., 2015) and Parkinson's disease (Gerhard et al., 2006).

Most TSPO ligands were synthesized primarily as neuroimaging agents and used as diagnostic tools for various neuroinflammation-related neuropathological conditions (Cagnin et al., 2004; Edison et al., 2008; Gerhard et al., 2006; Harberts et al., 2013; Ouchi et al., 2005; Pavese et al., 2006; Rissanen et al., 2014; Versijpt et al., 2005; Vowinckel et al., 1997; Yasuno et al., 2008; Zurcher et al., 2015). Some TSPO ligands also have therapeutic potential in neuroprotection, neuroregeneration and anxiety (Milenkovic et al., 2015; Rupprecht et al., 2010). Lots of in vitro studies have demonstrated that classical ligands of TSPO, PK11195 and Ro5-4864, could reduce microglia activation and have protective effects against neuroinflammation (Choi et al., 2011; Karlstetter et al., 2014; Mills et al., 2008; Veiga et al., 2007). Some in vivo studies have been attempted in reaffirming the in vitro data. Pretreatment

*Abbreviations:* Aβ, β-amyloid protein; AD, Alzheimer's disease; APP, amyloid precursor protein; BACE, β-site APP cleaving enzyme; BDNF, brain-derived neurophic factor; COX, cyclooxygenase; IDE, insulin-degrading enzyme; LPS, lipopolysaccharide; MWM, Morris water maze; SEM, standard error of the mean; TSPO, translocator protein 18 kDa.

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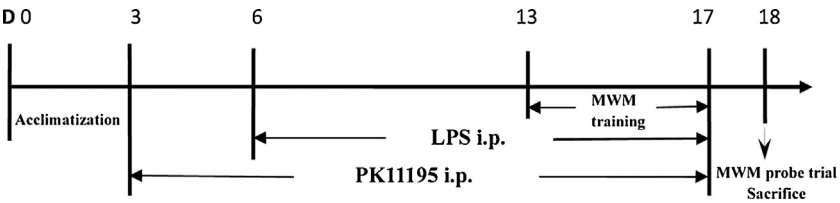


**Fig. 1.** Experimental design. PK11195 (3 mg/kg, i.p.) was administered consecutively in group LPS + PK11195 and PK11195. Three days later, LPS (500 μg/kg, i.p.) was given in group LPS and LPS + PK11195. Seven days after LPS administration, cognitive function was assessed by MWM. The drugs were not discontinued until the mice were sacrificed.

of Ro5-4864 or PK11195 prevented neuronal death and reactive gliosis in experimental models of excitotoxic and traumatic brain injury (Leaver et al., 2012; Ryu et al., 2005). Veiga et al., reported that PK11195 could attenuate inflammatory responses in the brain caused by intracerebroventricular infusion of the bacterial endotoxin lipopolysaccharide (LPS) in rats (Veiga et al., 2007). Studies investigating the effect of TSPO ligands on cognition and amyloidogenesis were rare (Daugherty et al., 2013). In a mouse model of AD, Ro5-4864 treatment not only reduced the development of AD-related pathology in adult mice, but also reversed neuropathology and improved cognitive function in aged 3xTgAD mice (Barron et al., 2013).

In this study, we adopted an animal model of neuroinflammation caused by chronic systemic administration of LPS. This model has been used to mimic pathophysiology of AD characterized by neuroinflammation, gliosis, β-amyloid proteins (Aβ) accumulation, cognitive dysfunction (Gong et al., 2010; Jaeger et al., 2009; Kahn et al., 2012; Kranjac et al., 2012; Lee et al., 2008). Though Barron et al., reported that Ro5-4864 could alleviate AD-related pathology in mice, PK11195 is different from Ro5-4864 in many aspects (Barron et al., 2013). PK11195 binds exclusively to TSPO, whereas Ro5-4864 requires other mitochondrial protein components for full binding capacity; Ro5-4864 is a full agonist of TSPO while PK11195 is a partial agonist of TSPO (do Rego et al., 2015; Zhang et al., 2014). Actually, PK11195 may display agonistic or antagonistic effects depending on the cell type, tissue microenvironment and/or the existence of an endogenous ligand (Totis et al., 1989). Here, we sought to evaluate the effect of PK11195 on LPS-induced chronic neuroinflammation and its subsequent pathology.

## 2. Materials and methods

### 2.1. Animals

Male SPF C57/BL6 mice (Beijing SPF Animal Technology Company, Beijing, China) (10–12 weeks old weighing 25 g ± 2 g) were housed in a light-, temperature-, and humidity-controlled habitat and were allowed free access to food and water. Experiments were conducted in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China (November 14, 1988) and the guidelines for care and use of laboratory animals from the institution's Animal Ethics Committee of Chinese PLA General Hospital.

### 2.2. Experimental design

Forty-eight mice were randomly assigned to four groups: Control ($n = 12$), LPS ($n = 12$), LPS + PK11195 ($n = 12$), PK11195 ($n = 12$). PK11195 (3 mg/kg, i.p.) was administered consecutively in group LPS + PK11195 and PK11195 once per day. Meanwhile, an equivalent volume of vehicle was given in group Control and LPS. Three days after PK11195 administration, the first injection of LPS (500 μg/kg, i.p.) was given 30 min after PK11195 administration in group LPS and LPS + PK11195 while an equivalent volume of sterile saline was given to mice in group Control and PK11195. LPS was also administered once per day. Seven days after LPS administration, the sickness behavior disappeared and locomotor activity returned to normal (Kahn et al., 2012), cognitive function was assessed by Morris water maze (MWM). The drugs were not discontinued until the mice were sacrificed. The outline of the treatment schedule and behavioral test is shown in Fig. 1.

LPS (Escherichia coli serotype 0111:B4) and PK11195 were purchased from Sigma–Aldrich (St Louis, MO, U.S.A.). LPS was dissolved in saline and PK11195 was dissolved in saline containing 0.4% Tween 80. Dose of LPS (500 μg/kg, i.p.) was chosen according to the results of our preliminary study and dose of PK11195 (3 mg/kg, i.p) was based on published literature (Barron et al., 2013).

### 2.3. Morris water maze (MWM)

The MWM apparatus is a large circular pool of water maintained at 22 °C with a fixed platform hidden just 1 cm below the surface of

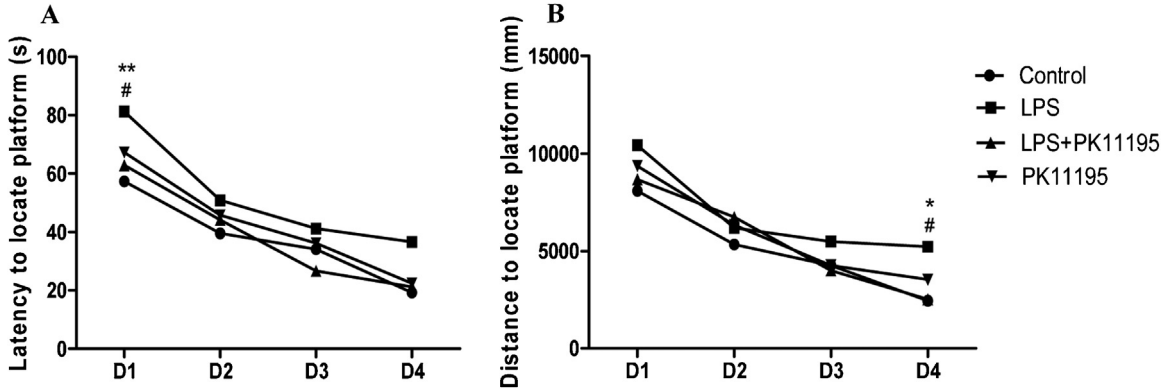


**Fig. 2.** PK11195 improved the LPS-induced learning dysfunction of mice in the acquisition phase of MWM. (A) Effect of LPS and PK11195 on latency to locate the platform (B) effect of LPS and PK11195 on distance to swim to locate the platform. Data are expressed as mean ($n = 10 - 12$). $^{*}p < 0.05$, $^{**}p < 0.01$ vs. Control; $\#p < 0.05$ vs. LPS.