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Research report

Over-expression of TSPO in the hippocampal CA1 area alleviates cognitive dysfunction caused by lipopolysaccharide in mice

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ABSTRACT

The translocator protein 18 kDa (TSPO) is closely related to regulation of immune/inflammatory response. However, the putative role and signaling mechanisms of TSPO in regulation of neuroinflammation remain unclear. GV287 lentiviral vectors mediating TSPO over-expression were injected into bilateral hippocampal CA1 areas to test whether TSPO over-expression was neuroprotective in lipopolysaccharide (LPS)-induced mice model. Finasteride, a blocker of allopregnanolone production, was used to test whether the protective effects were related to steroideogenesis. The results demonstrated that TSPO over-expression increased progesterone and allopregnanolone synthesis. TSPO over-expression in CA1 area improved LPS-induced cognitive deficiency in mice and this cognitive improvement was reversed by finasteride administration. These data suggest that up-regulation of TSPO level during neuroinflammation may be an adaptive response mechanism, a way to provide more neurosteroids. We confer that TSPO could be an attractive drug target for controlling neuroinflammation in the future.

1. Introduction

The translocator protein 18 kDa (TSPO), previously known as the peripheral benzodiazepine receptor (PBR), is a five transmembrane protein mainly found in steroid-synthesizing tissues, including the nervous system (Braestrup et al., 1977; Braestrup and Squires, 1977). TSPO is primarily localized in the outer mitochondrial membrane and is associated with several cellular functions, including regulation of cholesterol transport, biosynthesis of steroids, immunoregulation, modulation of mitochondrial functions, anion transport, cell proliferation and apoptosis (Jaremko et al., 2015; Midzak et al., 2015; Rupprecht et al., 2010).

TSPO in the nervous system is predominantly expressed in glial cells (microglia and astrocytes) and is closely related to regulation of immune/inflammatory response. In acute inflammatory conditions, glial cells were activated, TSPO expression was up-regulated (Dickens et al., 2014; Liu et al., 2014). In degenerative diseases with chronic neuroinflammation, e.g. Alzheimer's disease (AD) (Edison et al., 2008; Yasuno et al., 2008), fronto-temporal dementia (Cagnin et al., 2004), multiple sclerosis (Versijpt et al., 2005), Huntington's disease (Pavese et al., 2006), amyotrophic lateral sclerosis (Turner et al., 2004) and Parkinson's disease (Gerhard et al., 2006), TSPO levels were strongly up-regulated at the sites of degenerative changes, which were shown by TSPO ligand binding imaging. TSPO ligands were also proved to own neuroprotective effects in numerous in vitro and in vivo studies (Barron et al., 2013; Zhao et al., 2011). However, the putative role and signaling mechanisms of TSPO in regulation of neuroinflammation remain unclear.

As one of the most well-characterized functions, TSPO is involved in the rate-limiting step in neurosteroids production, i.e. translocation of cholesterol from the outer to the inner mitochondrial membrane. Neurosteroids include progesterone, allopregnanolone, oestradiol, testosterone, pregnenolone, pregnenolone sulphate, allotetrahydrodeoxycorticosterone, dehydroepiandrosterone and dehydroepiandrosterone sulphate (Borowicz et al., 2011). As demonstrated in experimental models and clinical studies, some neurosteroids, especially allopregnanolone, concentrations in the

Abbreviations: AD, Alzheimer's disease; EGFP, enhanced green fluorescence protein; GABA, γ-aminobutyric acid; HSD, hydroxysteroid dehydrogenase; LPS, lipopolysaccharide; MWM, Morris water maze; PBR, peripheral benzodiazepine receptor; PVDF, polyvinylidene fluoride; SEM, standard error of the mean; TSPO, translocator protein 18 kDa

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brain have been reported to be markedly altered after hypoxia (Billiards et al., 2006), infection, LPS treatment (Billiards et al., 2002; Ghezzi et al., 2000), injury (Labombarda et al., 2006; Meffre et al., 2007), or other stresses (Liu et al., 2012). Exogenous progesterone and allopregnanolone confer anti-inflammatory effects and neuroprotection in many injury models, including endotoxin injury models (Ghezzi et al., 2000; Goddard et al., 2013; Lei et al., 2014), cerebral ischemic stroke (Yousuf et al., 2014), traumatic brain injury (Robertson et al., 2015) and AD (Chen et al., 2011; Wang et al., 2005; Wang et al., 2010). Allopregnanolone is reported as the only small molecule that not only promotes neuroregeneration but also reduces AD pathology burden, and now is considered as a potential AD's therapeutic drug (Irwin et al., 2011). In view of these evidences, neurosteroid mechanism has been implicated as an adaptive mechanism following brain damage (Garcia-Ovejero et al., 2005) and TSPO up-regulation in the pathological status is supposed to meet the organism's need for more neurosteroids.

We designed the present study to investigate the underlying effects of TSPO in neuroinflammation and the mechanisms. LPS-induced mice model was used to test if TSPO over-expression in hippocampal CA1 area was protective for cognitive dysfunction. In this animal model (Chen et al., 2008; Kahn et al., 2012; Lee et al., 2008), neuroinflammation was induced by systemic LPS administration through neural and humoral mechanisms (Steinman, 2010). Finasteride was adopted as a tool in our study to block the production of allopregnanolone and test whether the neuroprotective effects of TSPO over-expression were related to steroideogenesis (Fig. 1).

2. Results

2.1. Optimization of dose of finasteride

Results of 2-way ANOVA analysis indicated the existence of significant main effects of time interval ($F_{time}=59.43$, $P<0.0001$) and dose ($F_{dose}=7.319$, $P=0.0023$). No significant interaction between the two factors ($F_{time\times dose}=0.2402$, $P=0.8671$) was observed. Post hoc analysis suggested that 5 mg/kg finasteride did not significantly influence locomotor activities of the mice during the 1st and 2nd 5-min session while all the higher doses did (Fig. 2). Thus, dose of 5 mg/kg finasteride was adopted in further experiment.

2.2. Lentivirus mediating TSPO over-expression (TSPOoe (+) lentivirus) up-regulates TSPO expression in vitro and in vivo

Fluorescent microscopy suggested that HEK 293 cells were successfully transfected by lentivirus, as indicated by enhanced green fluorescence protein (EGFP)-positive cells (Fig. 3(A)). Cells transfected by TSPOoe (+) lentivirus increased TSPO expression by 6 folds, when compared with control cells (cells with no lentivirus transduction) and TSPOoe (−) lentivirus (the GV287 lentiviral vectors that contained the non-targeting negative control sequence) -treated cells ($P<0.05$) (Fig. 3(B)).

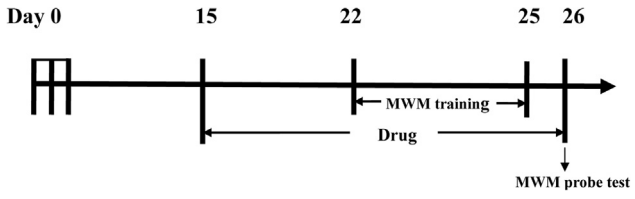

Fig. 1. Schedules of drug treatments and tests. Lentivirus (TSPOoe (−) or TSPOoe (+)) was microinfused into bilateral hippocampal CA1 areas on day 0. LPS, finasteride or vehicle was administered (i.p.) once a day from day 15 until the end of behavioral tests (day 26). MWM training or testing was conducted 22 days after the microinfusions, precisely 1 h after finasteride/vehicle treatment.

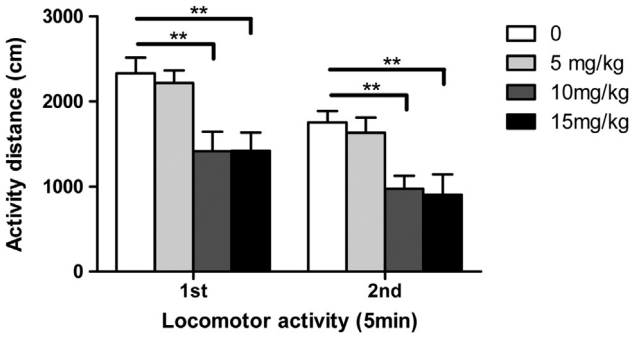

Fig. 2. Effect of finasteride dose on locomotor activity. One hour after finasteride administration (0, 5, 10, 15 mg/kg), locomotor activity of the mice was evaluated for the duration of 10 min. The results were demonstrated as the mice's activity distances in 5-min time intervals. $^{**}P<0.01$. Values shown are means ± SEM; n=6.

In vivo, the lentivirus-mediated transduction was traced by the specific expression of EGFP (Fig. 4(A) and (B)). The level of TSPO was evaluated by Western-blotting (Fig. 4(C)). Three-way ANOVA was performed to assess the effects of TSPOoe, LPS and finasteride on the expression of TSPO. There were significant main effects of TSPOoe ($F_{TSPOoe}=49.39$, $P=0.0001$) and LPS ($F_{LPS}=6.4$, $P=0.0184$), but not finasteride ($F_{finasteride}=1.58$, $P=0.2209$). The results suggested that TSPOoe (+) lentivirus injection increased the expression of TSPO significantly compared with the TSPOoe (−) lentivirus treatment ($P<0.001$) and LPS increased TSPO expression in injected area ($P<0.05$) (Fig. 4(D) and (E)) while finasteride had no effect on TSPO expression.

2.3. TSPOoe (+) lentivirus injection improves LPS-induced cognitive deficiency in Morris Water Maze (MWM), finasteride counteracts this effect

Fig. 5 summarizes the performance of each group during the acquisition phase of MWM. Repeat-measures multi-way ANOVA revealed that the animals' performances had improved over time, and the latency to locate platform seemed to be affected by time and LPS ($F_{time}=157.4$, $P=0.0001$; $F_{LPS}=10.53$, $P=0.0017$) (Fig. 5(A)). Swimming distance to locate platform was only affected by time ($F_{time}=184.96$, $P=0.0001$) (Fig. 5(B)). Post hoc analysis indicated that, to locate the platform, mice in LPS group needed significantly longer time than those in the Control group on Day 2 of training ($P<0.05$). Notably, there was no difference in the latency to locate platform between Control group and other groups on Day 4, which suggested that all mice had learned the task at the end of the acquisition phase. The fact that swimming speed was only affected by time ($F_{time}=6.01$, $P=0.0006$) suggested that mobility of the mice was not affected by either LPS or finasteride (Fig. 5(C)).

In the probe trial, longer duration of time spent in the target quadrant indicates better cognitive function. Three-way ANOVA revealed that there were significant main effects of TSPOoe ($F_{TSPOoe}=20.88$, $P=0.0001$) and LPS ($F_{LPS}=20.11$, $P=0.0001$) on duration of time spent in the target quadrant. A significant factor interaction between TSPOoe and finasteride ($F_{TSPOoe\times finasteride}=4.3$, $P=0.0413$) as well as an interaction among TSPOoe, LPS and finasteride ($F_{TSPOoe\times LPS\times finasteride}=5.85$, $P=0.0178$) (Fig. 6(A)) were observed. Post-hoc analysis suggested that mice in LPS group had significantly shorter duration in target quadrant than those in Control group and TSPOoe+LPS group. Additionally, mice in TSPOoe+LPS+FN group spent less time in the target quadrant than those in TSPOoe+LPS group and TSPOoe+FN group. No significant differences were reached in results of number of platform crossings