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Research Report

The molecular mechanism and effect of cannabinoid-2 receptor agonist on the blood–spinal cord barrier permeability induced by ischemia-reperfusion injury

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ABSTRACT

Previous studies have shown that modulation of the receptor-mediated endocannabinoid system during ischemia injury can induce potent neuroprotective effects. However, little is known about whether cannabinoid-2 (CB2) receptor agonist would produce a protective effect on blood–spinal cord barrier (BSCB) during ischemia. Using an *in vivo* transient spinal cord ischemia model in rats, JWH-015 (1 mg/kg, i.p.), a CB2 receptor selective agonist, or vehicles were injected 20 min before ischemia. The effects of JWH-015 on BSCB permeability, the major structural protein for the formation of caveolae, caveolin-1 (cav-1), tight junction (TJ) protein Occludin and zona occludens protein-1 (ZO-1) were examined at day 1, day 3 and day 7 of reperfusion after transient spinal cord ischemia in rats. Here we demonstrated that JWH-015 significantly down-regulated the expression of cav-1, up-regulated the expression of TJ proteins, and then decreased the permeability of BSCB compared with control group. In addition, using an *in vitro* BBB model, oxygen glucose deprivation (OGD) was applied to simulate spinal cord ischemia *in vitro* in Human brain microvascular endothelial cells (HBMECs). JWH-015 greatly increased the transepithelial electrical resistance (TEER) and changed the distribution of ZO-1 and Occludin. Moreover, JWH-015 induced the expression of p-PKB and p-FoxO1 protein and decreased the expression of cav-1, which were greatly reversed by ROS inhibitor or PI3K inhibitor. Taken together, all of these results suggested that JWH-015 might regulate the BSCB permeability and this effect could be related to paracellular and transcellular pathway. And pharmacological CB2R ligands offer a new strategy for BSCB protection during ischemic injury.

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1. Introduction

Spinal cord ischemia reperfusion injury (SCII) is a kind of severe injury from central nervous system. SCII is not only acute neurodegenerative diseases, but also could alter blood–spinal cord barrier (BSCB) function eventually lead to impaired neuronal signaling and cognition (Li et al., 2015). Therefore, studies on the regulatory mechanism of BSCB after SCII will provide new ideas for the prevention of ischemic diseases.

Evidence from basic pharmacological studies has shown that endocannabinoid system has been under investigation as a potential target for neuroprotection and it could protect against ischemic stroke (England et al., 2014). Studies demonstrate that it plays an important modulatory role in normal blood brain barrier (BBB) physiology, and also affords a protection to the BBB during ischemic stroke (Hind et al., 2015). Recently, a possible connection between the cannabinoid receptors (CB receptors) and the BBB also first gained attention. Hind et al. (2015) demonstrated that endocannabinoids modulate human BBB permeability *in vitro*. Pharmacological data have indicated that CB2 agonists are neuroprotective in cerebral ischemia (Choi et al., 2013) and increased transendothelial electrical resistance and the amount of tight junction protein present in membrane fractions during neuroinflammation (Ramirez et al., 2012), suggesting that CB2 receptor activation holds limitless promise as a practical and therapeutic intervention. However, it is unclear by which mechanism and the effect of CB2 receptor activation on BSCB during ischemia-reperfusion injury.

Caveolin-1, the major structural protein required for the formation of caveolae, is also highly necessary in regulating the activity and localization of signal molecules that are involved in vesicle fission (Mougeolle et al., 2015). The study confirmed that caveolin-1 are closely involved in the regulation of the permeability of BBB and directly regulate the expression of TJ protein and the number of pinocytosis vesicles (i.e. the paracellular pathway and transcellular pathway) (Zhao et al., 2014). In several models of adult brain injuries, the level of expression of caveolin-1 protein is increased in the endothelium in several animal models such as cold cortical injury (Nag et al., 2007), brain ischemia (Huang et al., 2012) and experimental spinal cord injury (Shin, 2007). And an increase of caveolin-1 in some brain injury models was related to BBB/BSCB breakdown (Nag et al., 2007). Altered expression of these TJ proteins could cause BBB breakdown and then result in edema formation after brain injury (Blixt et al., 2015). Both Occludin and ZO-1 are organized within the TJ by association with caveolin-1 in detergent-insoluble glycolipid rafts, membrane specializations closely related to caveolae. Caveolin-1 appears to constitute an early and critical modulator that leads to the disruption of TJ (Zhong et al., 2008). To investigate the mechanism and effect the CB2 receptor agonist pretreatment on BSCB after SCII, we utilized *in vivo* and *in vitro* ischemia model and evaluated the changes of BSCB permeability, the expression of TJ proteins, caveolin-1 protein, the level of FoxO1 phosphorylation and PKB phosphorylation in different time point group.

2. Results

2.1. JWH-015 pretreatment improved the permeability of BSCB in SCII model in rats

In the present study, the permeability of BSCB was evaluated by Evans blue leakage. The spinal cord tissue after SCII was stained in blue. Evans blue content and scope of blue staining in the spinal cord tissue was significantly decreased by JWH-015 pretreatment compared with SCII group (Fig. 1A). The Evans blue content of spinal cord tissue at day 1, day 3 and day 7 after SCII is 4.8 ± 0.4, 4.2 ± 0.5, and 3.5 ± 0.2, respectively. And they are 3.0 ± 0.3, 2.8 ± 0.2, and 2.5 ± 0.2 in JWH-015 group at day 1, day 3 and day 7 after SCII, respectively. There is a strong red fluorescence surrounding the vascular of in gray matter of spinal cord after SCII, which could be significantly reduced by JWH-015 pretreatment (Fig. 1B–F).

2.2. JWH-015 pretreatment prevented the alteration of Occludin, ZO-1 and Caveolin-1 induced by SCII in rats

The expression of TJ proteins Occludin and ZO-1 was measured by western blots at different time point after SCII in rats. The results showed that the expression levels of TJ proteins Occludin and ZO-1 in spinal cord tissue were significantly decreased at 1 day, 3 day and 7 day after reperfusion (Fig. 2A). Moreover, JWH-015 partially prevented the down-regulation of Occludin and ZO-1 induced by SCII (Fig. 2A). The IDV ratio of Occludin for western blots at day 1, day 3 and day 7 after SCII is 0.2 ± 0.03, 0.4 ± 0.04, 0.3 ± 0.042 (Fig. 2B and C). And the IDV is 0.8 ± 0.07, 0.85 ± 0.09, 0.9 ± 0.1 at day 1, day 3 and day 7 after SCII with JWH-015 pretreatment. And there was no significant difference in Sham-operated group and JWH-015+Sham-operated group at day 1, day 3 and day 7 (Fig. 2D–F). In addition, the expression and location of TJ proteins Occludin and ZO-1 were evaluated by immunohischemistry at different time point after SCII in rats. The effects of SCII-induced reduction of Occludin and ZO-1 in the vascular of the gray matter were attenuated by pretreatment with JWH-015 at day 1, day 3 and day 7 after reperfusion (Figs. 3 and 4). The mean optical density in SCII group and JWH-015+SCII group is shown in Figs. 3G and 4G. In addition, Western blot analysis showed that the expression level of caveolin-1 protein in spinal cord tissue was significantly induced at day 1, day 3 and day 7 after SCII (Fig. 5). Moreover, JWH-015 pretreatment greatly prevented the up-regulation of caveolin-1 induced by SCII (Fig. 5). The IDV ratios of caveolin-1 for western blot are shown in Fig. 5B and D.

2.3. JWH-015 pretreatment improved the permeability of in vitro BBB

In the study, the permeability of BBB *in vitro* was measured by TEER assays and HRP flux. OGD time-dependently reduced TEER across a monolayer of HBMECs. Furthermore, this effect of OGD was prevented by incubation of the cells with the JWH-015 for 20 min before exposure to OGD. The effects of JWH-015 on TEER were more significant in 10 μM and 100 μM groups than in the 1 μM group (Fig. 6A, $^{\#}P<0.05$). JWH-015 at the dose