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Original article

Plant-derived triterpene celastrol ameliorates oxygen glucose deprivation-induced disruption of endothelial barrier assembly via inducing tight junction proteins

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ABSTRACT

Background: The integrity and functions of blood-brain barrier (BBB) are regulated by the expression and organization of tight junction proteins.
Objective: The present study was designed to explore whether plant-derived triterpenoid celastrol could regulate tight junction integrity in murine brain endothelial bEnd3 cells.
Methods: We disrupted the tight junctions between endothelial bEnd3 cells by oxygen glucose deprivation (OGD). We investigated the effects of celastrol on the permeability of endothelial monolayers by measuring transepithelial electrical resistance (TEER). To clarify the tight junction composition, we analyzed the expression of tight junction proteins by RT-PCR and Western blotting techniques.
Results: We found that celastrol recovered OGD-induced TEER loss in a concentration-dependent manner. Celastrol induced occludin, claudin-5 and zonula occludens-1 (ZO-1) in endothelial cells. As a result, celastrol effectively maintained tight junction integrity and inhibited macrophage migration through endothelial monolayers against OGD challenge. Further mechanistic studies revealed that celastrol induced the expression of occludin and ZO-1) via activating MAPKs and PI3K/Akt/mTOR pathway. We also observed that celastrol regulated claudin-5 expression through different mechanisms.
Conclusion: The present study demonstrated that celastrol effectively protected tight junction integrity against OGD-induced damage. Thus, celastrol could be a drug candidate for the treatment of BBB dysfunction in various diseases.

Introduction

Blood-brain barrier (BBB) is a well-organized physiological structure involving endothelial cells, astrocytes, microglia, and neurons for the maintenance of CNS homeostasis (Daneman, 2012; Hawkins and Davis, 2005). Cerebral endothelial cells are linked together through the interactions between multiple tight junction proteins including claudins, occludin and junctional adhesion molecules to separate blood and CNS tissues (Wolburg and Lippoldt, 2002). Tight junction integrity is also regulated by cytoskeleton adaptor proteins including ZO-1 inside the cells (Gonzalez-Mariscal et al., 2003). BBB disruption is implicated in the pathogenesis of various neurodegenerative diseases such as ischemic stroke and Alzheimer's disease (Burgess et al., 2014; Shah and Abbruscato, 2014). For example, hypoxia induces the loss of tight junction integrity and subsequently disrupts BBB (Hawkins and Davis, 2005). Recent studies have demonstrated that ischemia reperfusion decreases the expressions of tight junction proteins such as occludin, claduin-5 and ZO-1, while increases BBB permeability (Engelhardt et al., 2014; Liu et al., 2014; Wang et al., 2013). Therefore, tight junction is recognized as an important therapeutic target for restoring BBB integrity and alleviating brain damage.

Botanical compounds including curcumin, baicalin and ginsenoside Rg1 are recently recognized for the therapeutic potential to recover BBB integrity in cultured endothelial cells or in rat model of cerebral ischemia reperfusion (Wang et al., 2013; Zhou et al., 2014; Zhu et al., 2012). Along this line, celastrol (Fig. 1(A)) is a pentacyclic triterpene initially isolated from Chinese medicine herb *Tripterygium Wilfordii* Hook F. (T. wilfordii), a member of Celastraceae family. Previous studies suggest that celastrol is a potent anti-inflammatory, anti-cancer and neuroprotective drug candidate (Chen et al., 2014; Jung et al., 2007; Shrivastava et al., 2015). It was recently shown that celastrol protected the brains in a rat model of cerebral ischemia injury via downregulating the expression of

Abbreviations: CNS, central nervous system; MAPKs, mitogen-activated protein kinases; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; PI3K, phosphoinositide 3-kinase; mTOR, mechanistic target of rapamycin.

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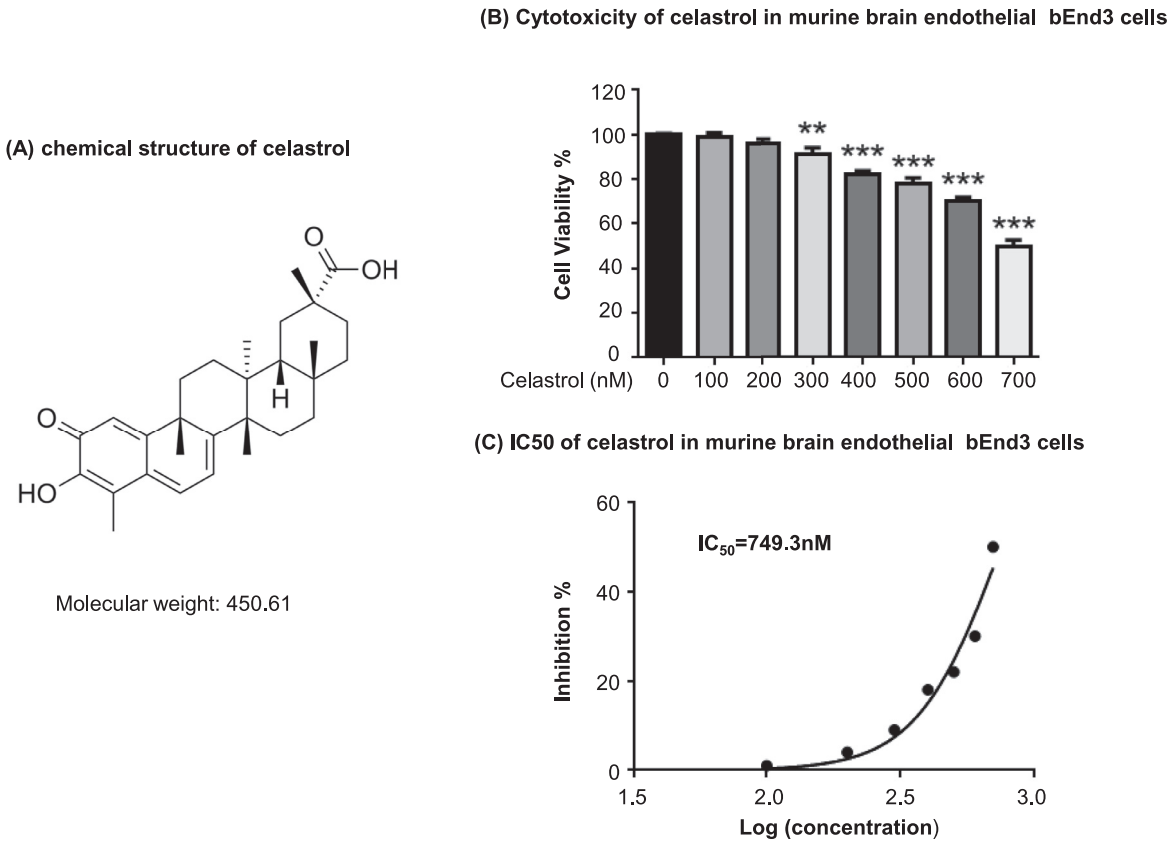

Fig. 1. Effects of celastrol on the cell viability of murine brain endothelial bEnd3 cells. (A) Chemical structural of celastrol. (B) Cell viability assay. Endothelial bEnd3 cells were treated with celastrol at indicated concentrations for 72 h. The cell viability was determined by a colorimetric MTT assay while the untreated controls were defined as a viability of 100%. The results were presented as mean ± SD of three independent experiments. **, $p < 0.01$; ***, $p < 0.001$ (Celastrol vs Control). (C) Calculation of IC_{50} value. The IC_{50} value of celastrol was calculated from the results of MTT assay in Fig. 1(B) with GraphPad Prism software (La Jolla, CA, USA).

p-JNK, p-c-Jun and nuclear factor NF-κB (Li et al., 2012). This study suggests that celastrol may regulate tight junction integrity to support BBB functions.

In the present study, we investigated the effect of celastrol on the expression of occludin, claudin-5 and ZO-1, and BBB permeability in murine endothelial cell line bEnd3 after oxygen-glucose deprivation (OGD) challenge. We further explored the molecular mechanisms by focusing on MAPKs (e.g., p38, ERK1/2, JNK) and PI3K/Akt/mTOR pathway.

Materials and methods

Antibodies and biochemical reagents

Antibodies against p38, ERK1/2, JNK, Akt, mTOR, p70S6K, GAPDH, phospho-p38, phospho-ERK1/2, phospho-JNK, phospho-Akt, phospho-mTOR, phospho-p70S6K were purchased from Cell Signaling Technology (Boston, MA, USA). Antibodies against occludin, claudin-5 and ZO-1 were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Anti-rabbit HRP-conjugated IgG secondary antibody was purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's Medium (DMEM), fetal bovine serum (FBS), 100x L-glutamine and 100x penicillin and streptomycin solution were purchased from Invitrogen (Carlsbad, CA, USA). Protein Assay Dye Reagent Concentrate was purchased from Bio-Rad (Hercules, CA, USA). Celastrol with the purity of over 98% (HPLC) was purchased from Nanjing Spring and Autumn Biological Engineering Co., Ltd. (Nanjing, China). Other chemical inhibitors were purchased from Selleck (Houston, TX, USA)

Cell culture

Mouse brain endothelial cell line bEnd3 and macrophage cell line RAW264.7 were obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in DMEM supplemented with 10% FBS, 1% L-glutamine and 1% penicillin and streptomycin at 37 °C in a humidified incubator containing 5% CO_2.

Measurement of cell viability

The cell viability was evaluated by a standard colorimetric assay using 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) as previously described (Yang et al., 2014). After celastrol treatment at the indicated concentrations for 72 h, the cell monolayers were incubated with 0.5 mg/ml MTT in phosphate-buffered saline (PBS) for 4 h. Formazan production was determined by measuring the absorbance at 570 nm on a Bio-Rad microplate reader (Hercules, CA, USA). The cell viability was presented as a percentage relative to vehicle-treated controls.

Oxygen-glucose deprivation (OGD) challenge

OGD challenge was performed essentially as described (Qi et al., 2012). The bEnd3 cells were incubated in deoxygenated and glucose-free DMEM medium. The cells were moved into a plastic hypoxic chamber (BioSpherix, Lacona, NY, USA) under 95% N_2 and 5% CO_2 and at 37 °C for indicated times. Drug treatment was performed during subsequent incubation under normoxia conditions.