Trilobatin attenuates blood-brain barrier dysfunction induced by cerebral ischemia reperfusion by targeting MMP9: Involvement of APOE4/CypA/NF-κB signaling

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Abstract

Background and Purpose: Blood-brain barrier (BBB) breakdown is one of the most crucial pathological changes of cerebral ischemia-reperfusion (I/R) injury. Trilobatin (TLB), a naturally occurring food additive, exerts neuroprotective effect against cerebral I/R injury as demonstrated in our previous study. This study was designed to investigate the effect of TLB on disruption of BBB after cerebral I/R injury. Experimental Approach: Rats with focal cerebral ischemia caused by transient middle cerebral artery occlusion (MCAO) and brain microvascular endothelial cells along with human astrocytes to mimic blood brain barrier (BBB) injury caused by oxygen and glucose deprivation (OGD) followed by reoxygenation (OGD/R). Key results: The results showed that TLB effectively maintained the integrity of BBB and inhibited neuronal loss following cerebral I/R challenge. Furthermore, TLB dramatically increased tight junction proteins including ZO-1, occludin and claudin 5, as well as decreased the levels of apolipoprotein E (APOE) 4, cyclophilin A (CypA), and phosphorylated nuclear factor kappa B (NF-κB), thereby reduced proinflammatory cytokines. In addition, TLB also decreased Bax/Bcl-2 ratio and cleaved-caspase 3 level along with reduced the number of apoptotic neurons. Intriguingly, molecular docking and transcriptomics predicted MMP9 was a prominent gene evoked by TLB treatment. Furthermore, the protective effect of TLB on OGD/R-induced the loss of BBB integrity in human brain microvascular endothelial cell and astrocyte co-cultures in vitro was markedly reinforced by knockdown of MMP9. Conclusions and implications: Our findings reveal a novel property of TLB: saving BBB disruption following cerebral I/R via targeting MMP9 and inhibiting APOE4/CypA/NF-κB axis.

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Τριλοβατιν αττενυατεσβλοοδ-βραιν βαρριερ δψσφυνστιον ινδυχεδ βψ χερεβραλ ισχηεμια ρεπερφυσιον βψ ταργετινγ ΜΜΠ9: Ινολεμεντ οφ ΑΠΟΕ4/ˆψπΑ/ΝΦ-κΒ σιγναλινγ

Linying Feng[1,2], Yeli Li[1,2], Mu Lin[1,2], Dianyou Xie[1,2], Yunmei Luo[1,2], Nana Chen[2], Zhixu He[3], Qihai Gong[1,2], Yizhun Zhu[1], Jianmei Gao[1,2*]

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Author contribution

LYF, YLL, ML, and NNC performed the experiments. DYX and YML helped with bioinformatics analysis and molecular docking analysis. All authors were involved in analysis of data. LYF wrote the manuscript. JMG, QHG ,YZZ, and HZX design the experiments and revised the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

Declaration of transparency and scientific rigour

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the BJP guidelines for Design & Analysis, Immunoblotting and Immunochemistry and Animal Experimentation and as recommended by funding agencies, publishers and other organizations engaged with supporting research.

Data availability statement

The authors declare that data supporting the findings of this study are available within the article and it's Supplementary information (Supplementary SFig.1, 2). Uncropped Western blots are shown in Supplementary material. All relevant datasets used and/or analyzed in this study are available from the corresponding author upon reasonable request.

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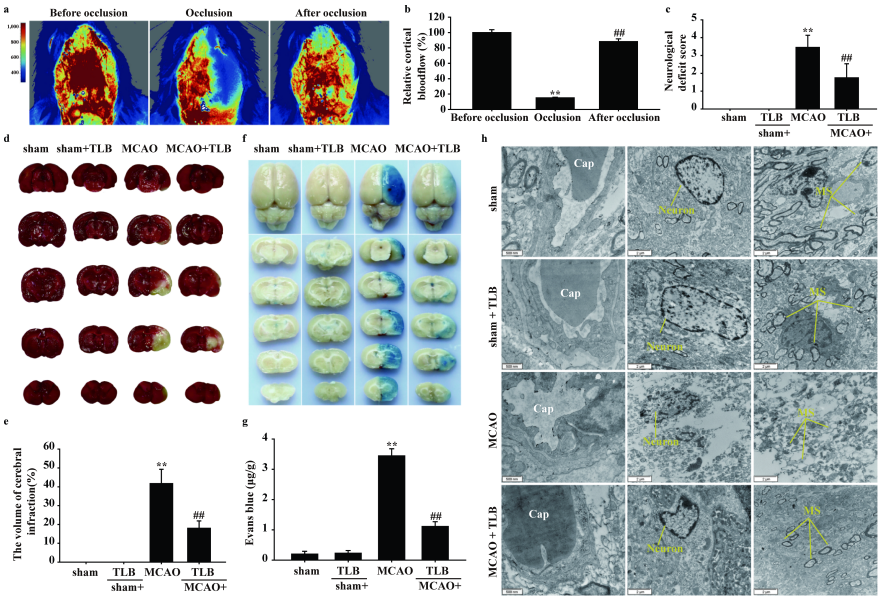
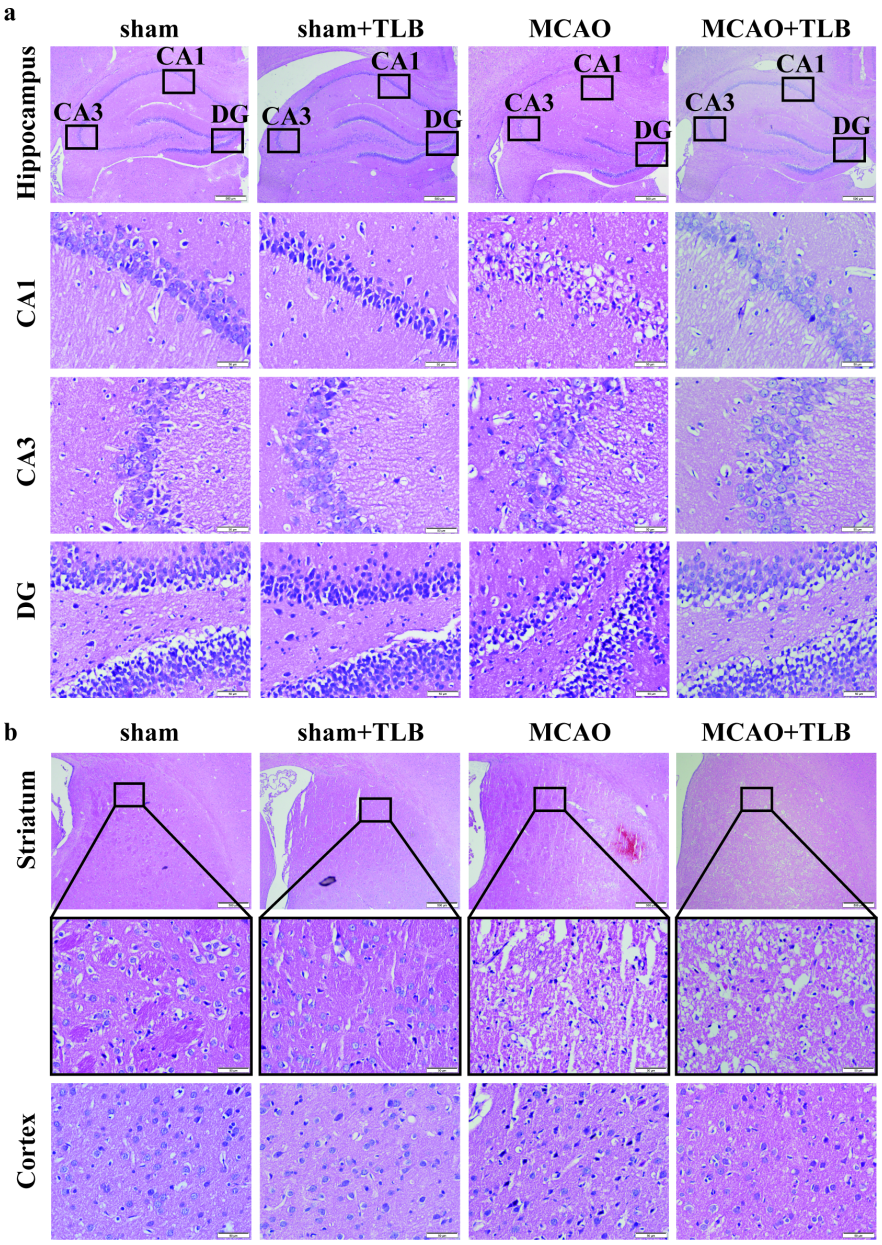
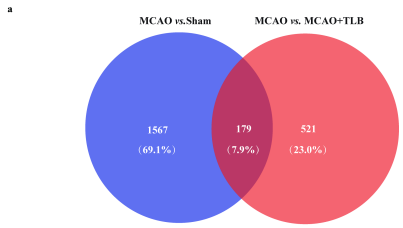
`Manuscript.docx` available at https://authorea.com/users/510398/articles/587532-trilobatin-attenuates-blood-brain-barrier-dysfunction-induced-by-cerebral-ischemia-reperfusion-by-targeting-mmp9-involvement-of-apoe4-cypa-nf-%CE%BAb-signaling



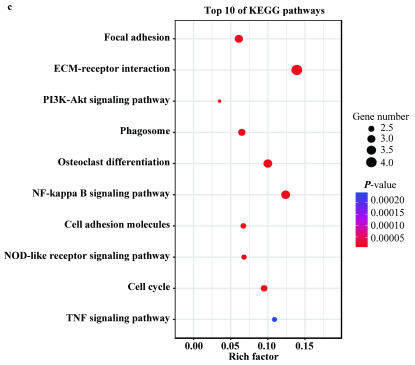


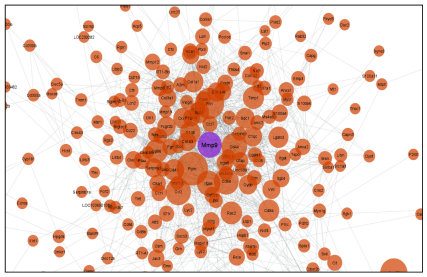


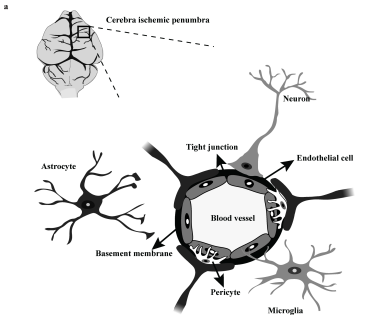
b

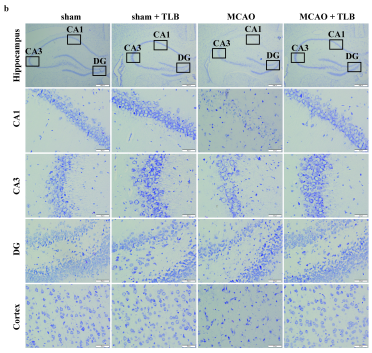
Biological processes in regulated by TLB in MCAO model

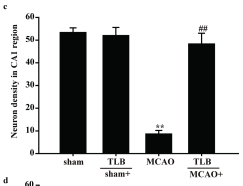
GO terms (biological processes)	*P*-value	Count
inflammatory response	2.50E-21	80
positive regulation of inflammatory response	1.50E-18	40
extracellular matrix organization	4.42252E-18	52
chromosome segregation	2.8167E-13	29
immune response	1.28764E-12	58
responsen to hypoxia	1.17251E-10	55
positive regulation of I-kappaB kinase/NF-kappaB signaling	7.78719E-10	41
lipopolysaccharide-mediated signaling pathway	7.80224E-10	18
apoptotic process	1.5388E-08	68
positive regulation of NF-kappaB transcription factor activity	1.78965E-08	35

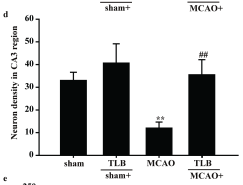


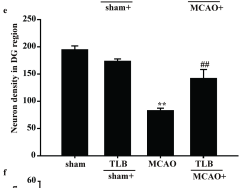


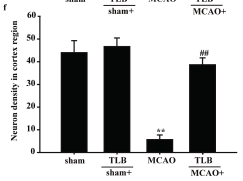


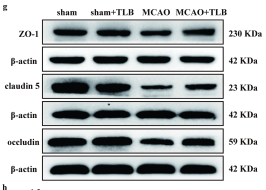


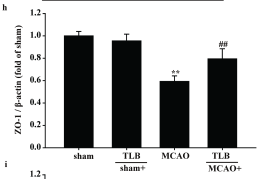


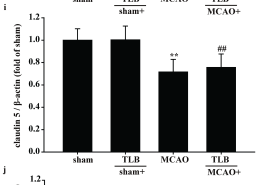


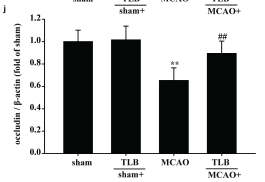


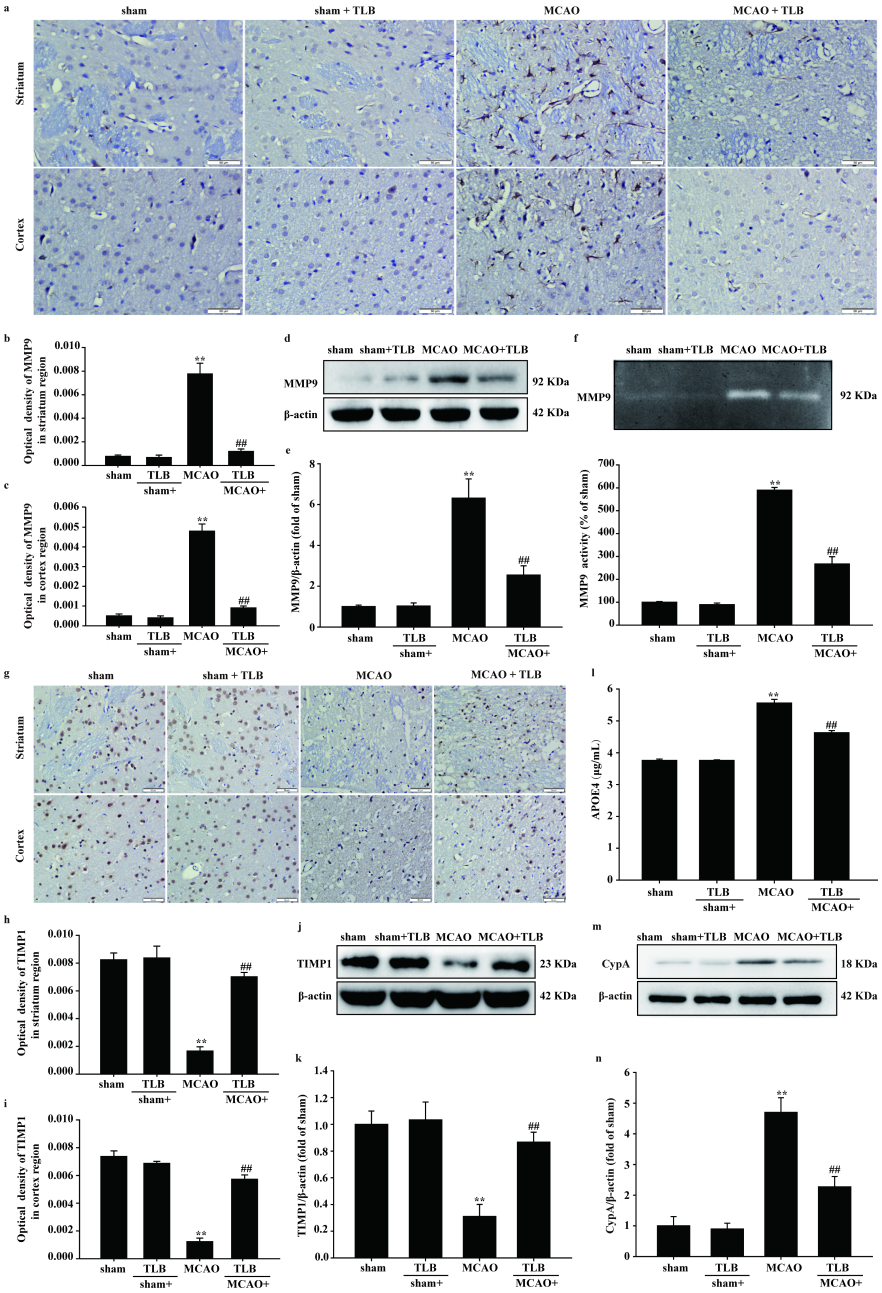


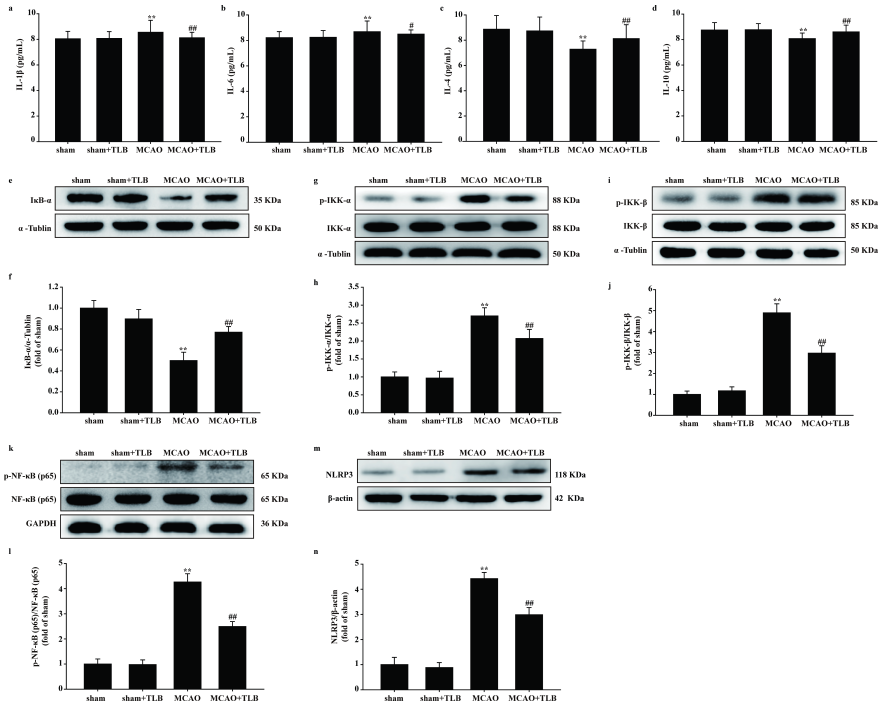


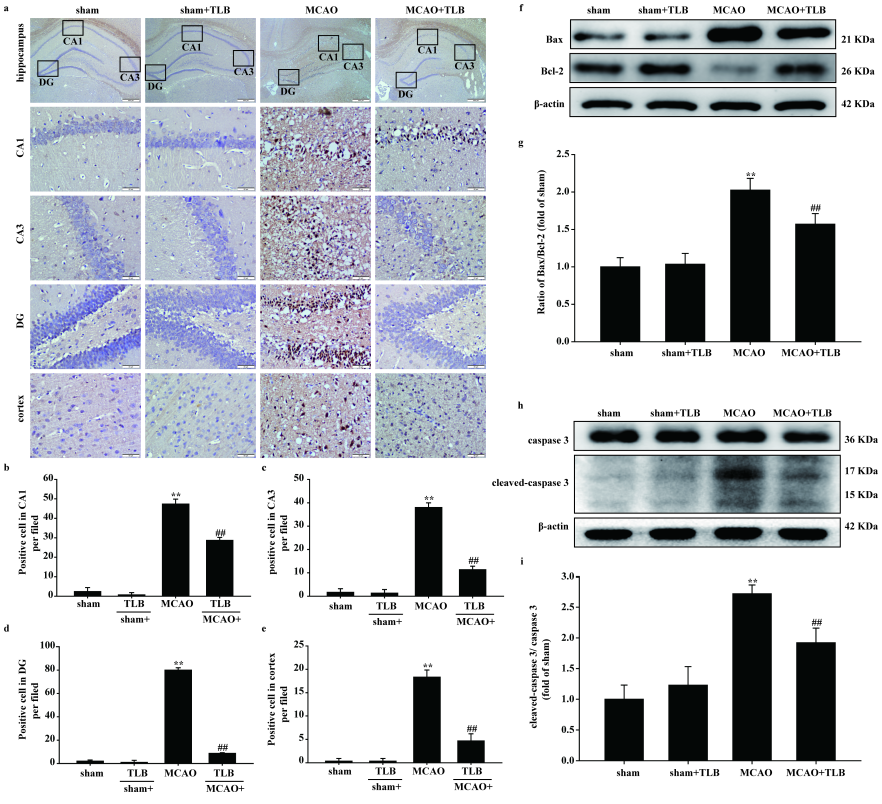


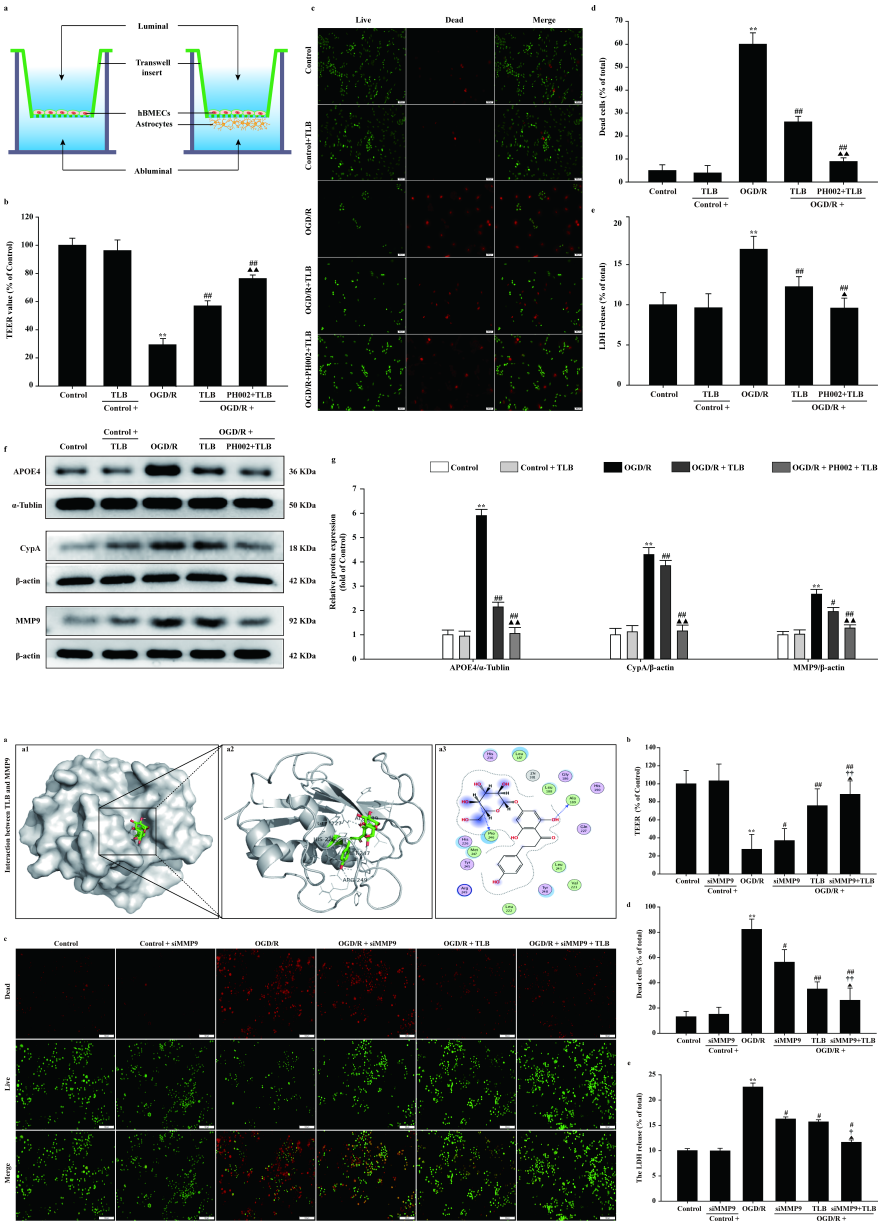


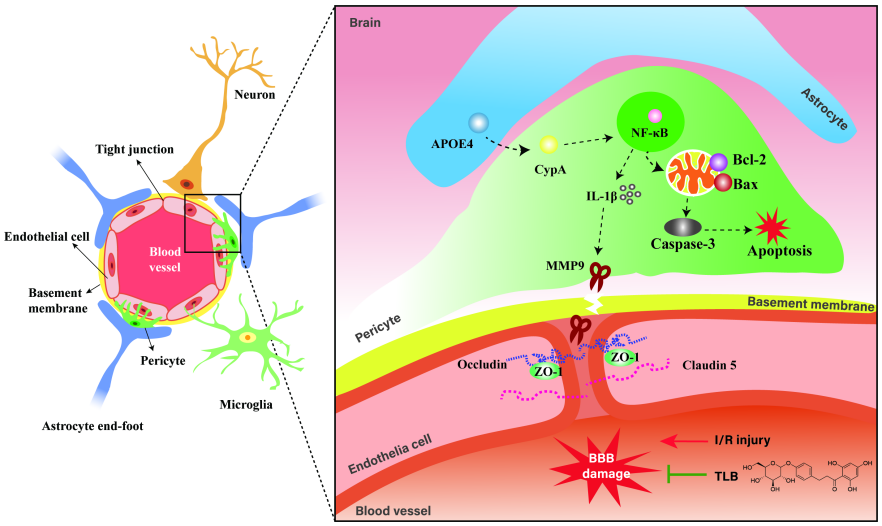




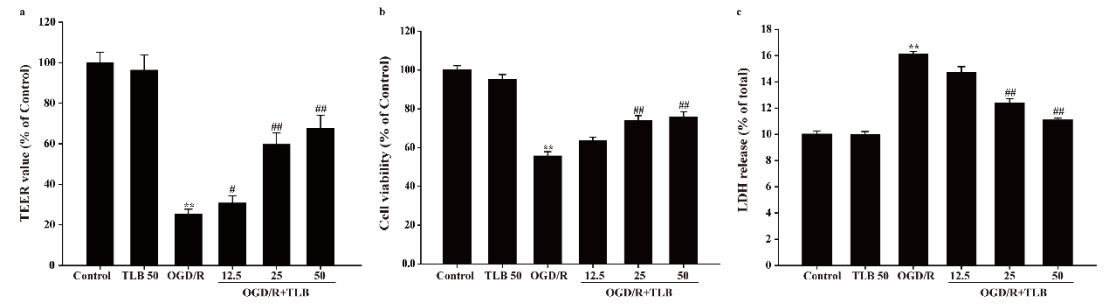




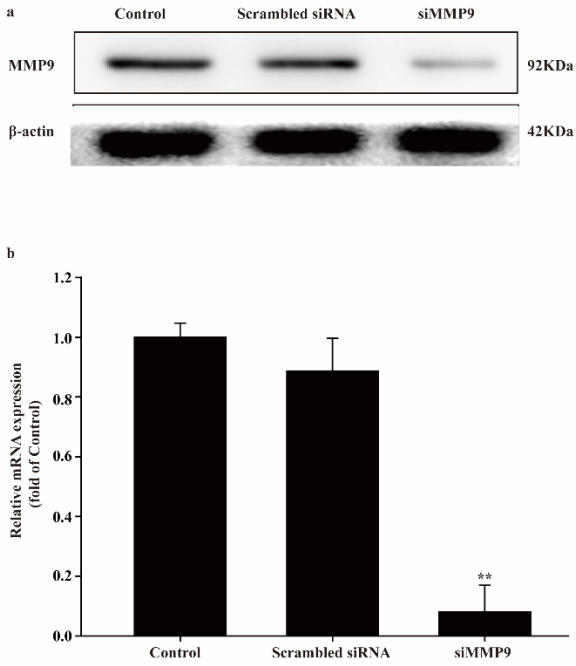




Supplementary Materials



Supplementary Fig. S1 Effects of TLB on BBB integrity breakdown induced by OGD 4 h/R 24 h in human BMEC/astrocytes co-cultures. Human BMEC/astrocytes were treated with TLB or without at different concentrations (12.5, 25, 50 μM) for 24 h. a. TEER value (n = 6). b. Cell viability (n = 6). c. LDH leakage (n = 6). Data are presented as mean ± SD. $^{**}P$<0.01 *versus* Control group; $^{\#}P$< 0.05, $^{\#\#}P$< 0.01 *versus* OGD/R group.



Supplementary Fig. S2 Knockdown of MMP9 by small interfering RNA (siRNA). a. The presentative western blot shown for MMP9 siRNA. b. Quantitation of MMP9. Data are presented as mean ± SD, $^{**}P$<0.01 *versus* Control group; n = 6 per group.

The BBB was impaired in the peri-infarct tissue after I/R injury. Furthermore, astrocytes regulate MMP9 expression in pericytes, and this regulation is mediated by astrocyte-derived apolipoprotein E (ApoE). Specifically, TLB, which in turn inhibits the proinflammatory cyclophilin A (CypA)/NF-kB/MMP9 pathway and reduces the degradation of TJs and BM proteins in the BBB in rats.