Triptolide Protects Neurons from Endoplasmic Reticulum Stress-Mediated Apoptosis in Cerebral Ischemic Injury Rats

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In this study, we explored whether the administration of T10 ameliorates cerebral I/R injury through the inhibition of ER stress. We further assessed whether the FADD/caspase-8/CHOP pathway is involved in the ER stress-induced apoptosis.

Materials and Methods

Animals

T e animal committee of Harbin Medical University approved all the experimental protocols and animal handling procedures. All experimental procedures and postoperative animal care were conducted in accordance with the national institute of health's guidelines for the care and use of laboratory animals. Adult male Sprague-Dawley rats (body weight, 250 g to 270 g) were purchased from vital river laboratory animal technology (Beijing, China). All rats were maintained on a 12 h light/dark cycle in a temperature room at 22℃ to 25℃ and allowed free access to food and water before surgery.

Experimental groups and drug administration

Statistical analysis

All data are expressed as mean ± SD. Statistical differences among the groups were evaluated by one-way ANOVA analysis, followed by Bonferroni test for the number of stained cells, paired t test for infarct volume, respectively. Statistical analysis was performed using Graph Pad Prism 6. Differences at $p<0.05$ were considered significant.

Results

T10 reduces reperfusion-induced infarct and neurological in rats

At 24 h after reperfusion, T10 treatment significantly reduced the infarct volume when compared with IC group ($P<0.05$). In addition, all rats subjected to MCAO showed neurological deficits over time to 24 h compared with placebo group; however, T10 treated group displayed significantly higher neurological scores than the rats only treated with MCAO ($P<0.05$). These results demonstrated that T10 ameliorated reperfusion-induced infarct and neurological deficits (Figure 1A and 1B).

T10 prevents ER stress-mediated expression of RyR, FADD, caspase-8 and CHOP in the peri-infarct area of rats

Immunohistochemistry analysis showed that there was no expression of FADD, caspase-8 and CHOP in placebo group. The immunoreactive cells for RyR were strongly positive in the peri-infarct area at 1 h after reperfusion. As the period of reperfusion increased, the number of RyR positive cells gradually reduced and very few remained at 24 h after reperfusion. However, in the core region, the expression of RyR rapidly disappeared after reperfusion. At 1 and 4 h after reperfusion, T10 pretreatment significantly reduced the number of RyR positive staining cells in the peri-infarct area compared with IC group (**$P<0.001$). Nevertheless, the expression levels of RyR in the peri-infarct area showed no statistical difference between IC group and T10 group at 24 h after reperfusion. The number of FADD-immunopositive cells increased dramatically from 1 h and peaked at 24 h of reperfusion in the ischemic periphery of the IC group, with no expression in the ischemic core. At 1 and 4 h after reperfusion, there was no statistical difference of the expression levels of FADD in the peri-infarct area between IC and T10 groups. However, compared with rats only treated with MCAO, the number of positive cells for FADD in the peri-infarct area significantly declined at 24 h after reperfusion in T10 group (**$P<0.001$). Thus, T10 decreased ER stress-induced expression of RyR and FADD in the peri-infarct area of rats (Figure 2A and 2B).

Both necrosis and apoptosis contribute to neuronal death from cerebral ischemia. Necrotic death occurs rapidly in the ischemic core region and apoptotic damage develops in the peri-infarct area afer MCAO [34,35]. We found that the expression of FADD, caspase-8 and CHOP was not detected in the ischemic core but were obvious in the peri-infarct area, which may be due to the rapid initiation of necrosis in the ischemic core. Moreover, we observed that the ER stress-associated factors were predominantly expressed in the ischemic penumbra, which is in agreement with previous reports that under severe ER stress, apoptotic pathways can be activated inducing the expression of downstream efectors [36-38]. Our fndings further revealed that T10 signifcantly down-regulated the expression levels of FADD, caspase-8, and CHOP in the peri-infarct area. However, T10 did not afect the expression of these factors in the central area afer reperfusion, suggesting that T10 may not efectively inhibit necrotic cell death. T erefore, we propose that the neuroprotective efect of T10 against infarct expansion is partly due to the suppression of ER stress-mediated apoptotic pathway in the peri-infarct area.

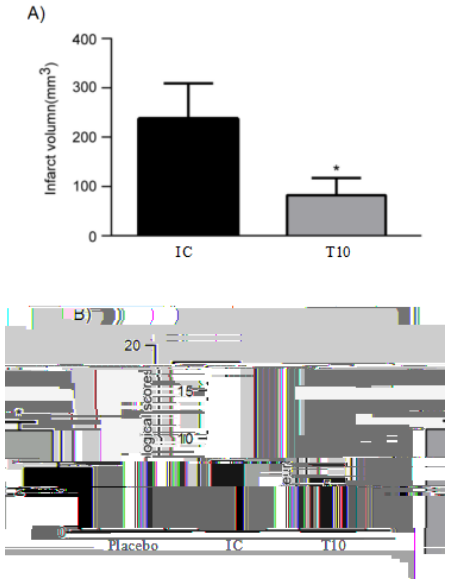

Figure 1: Treatment of rats with T10 led to reduced infarction volume and increased neurological scores afer reperfusion. (A) Quantitative analysis of infarct volume in rats at 24 h afer

upregulated at 1 h and peaked at 24 h after

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