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ORIGINAL ARTICLE

Neuroprotective Effects of Grape Seed Procyanidin Extract on Ischemia-Reperfusion Brain Injury△

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Key words: grape seed procyanidin extract; oxidative stress; neuroprotection; ischemia-reperfusion injury

Objective Oxidative stress (OS) plays a crucial role in ischemic stroke. Grape seed procyanidin extract (GSPE) was reported to be a critical regulator of OS. We hypothesized that GSPE might also be protective in ischemia-reperfusion brain injury. This study aimed to explore whether GSPE administration can protect mice from ischemia-reperfusion brain injury.

Methods Transient middle cerebral artery occlusion (MCAO) was conducted followed by reperfusion for 24 hours to make ischemia-reperfusion brain injury in mice that received GSPE (MCAOG, n=60) or normal saline (MCAONS, n=60). Sham-operated mice (GSPE group and normal saline group) were set as controls. The neurological severity score (NSS) was used to evaluate neural function impairment 1 hour, 24 hour, 3 days and 7 days after MCAO. Mice underwent brain T2WI imaging with a 3T animal MRI scanner 24 hours after reperfusion, and the stroke volume of brains were calculated according to abnormal signal intensity. Immunohistopathological analysis of brain tissues at 24 h after reperfusion was performed for neuronal nuclear antigen (NeuN), CD34, Bcl-2, and Bax. Glutathione peroxidation (GSH-Px) activity and the level of malonaldehyde (MDA) of brain tissue were also examined. The above indexes were compared among the groups statistically.

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Results Significant functional improvement was observed 24 hours after MCAO in MCAOG group compared to MCAONS group ($P<0.05$). MCAOG group had smaller cerebral stroke volume (22.46 ± 11.45 mm^3 *vs.* 47.84 ± 9.06 mm^3, $P<0.05$) than MCAONS group 24 hours after MCAO. More mature NeuN-immunoreactive neurons and more CD34-positive cells in peri-infarct zones were observed in brain tissue of MCAOG mice 24 h after MCAO than that of MCAONS mice (both $P<0.05$). MCAONS mice had significantly higher number of Bax-positive cells in brain tissue than MCAOG ($P<0.05$). The mean MDA level was significantly lower ($P<0.05$) and the GSH-Px activity was significantly higher ($P<0.05$) in brains of MCAOG mice compared to those of MCAONS mice.

Conclusion GSPE administration protects mice from ischemia-reperfusion brain injury through attenuating oxidative stress and apoptosis, promoting angiogenesis, and activating antioxidant enzyme GSH-Px. GSPE may represent a new therapeutical direction for the treatment of ischemia-reperfusion brain injury.

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STROKE is one of the most leading global causes of mortality in the world.[1] Even in patients with the same ischemia degrees, a large variability in neuronal functional recoveries exists. Therefore, it is vital to illuminate exact molecular biological mechanisms underlying the pathogenesis of ischemia-reperfusion brain injury and to explore more effective therapies.

Oxidative stress (OS) plays an important role in cardia-cerebrovascular diseases.[2] Many human and animal researches demonstrated a correlation between ischemia-reperfusion brain injury and increased systemic and local OS.[2, 3] Targeting OS in either primary ischemic-insult or the following reperfusion-injury, a lot of pharmacological drugs, particularly natural products derived from herbal medicine, are indicated to possess neuro-protective effect against ischemia-reperfusion brain injury.[4]

Grape seed polyphenol extracts (GSPEs) have strong anti-oxidant effects and can protect neurons and glia during ischemic-injury. GSPE contains a high concentration of flavonoids, linoleic acid fatty acids, and phenolic procyanidins. It has been said that the oxidant-lowering effect of GSPE is approximately fifty times higher than that of vitamin E and vitamin C. In a study by Prior *et al*, a dramatically increased post-prandial antioxidant capacity was observed after the consumption of mixed grape powder.[5] Nevertheless, the exact effect of GSPE on ischemia-reperfusion brain injury remains not clear. In our study, we explored whether mice that received GSPE could be protected from ischemia reperfusion-induced and OS mediated brain damage and function impairments. GSPE's effects on mitochondrial signaling pathways, which are crucially involve in OS processes, were also investigated.

MATERIALS AND METHODS

Animal preparation

Thirteen to 15-week-old C57 male mice were fetched from the animal house of Peking Union Medical College (Beijing, China). The mice were fed at a standard temperature (24°C–26°C) and an appropriate humidity and natural dark-light cycles for one week before the procedures to let them acclimate. All mice had free accesses to fresh food and water, and received humanized treatments according to the European Community guidelines for conducting experiments on animals.

Experimental grouping design

To evaluate the roles of GSPE in protecting from ischemia-reperfusion brain damage, a transient MCAO surgery[6] was done on both GSPE and normal saline treated mice to induce a focal ischemic stroke. Following a midline cervical incision, a 6-0 silicone-coated filament was introduced into the left common carotid artery and advanced into the internal carotid artery (ICA) for 9-12 mm from the common carotid bifurcation. The thread was left in place for 60 min to block the blood flow, and then removed to allow reperfusion.

The animals were divided randomly into four groups as follows: (a) sham-operated GSPE (SOG) group: pretreated with GSPE 50 mg/kg by intraperitoneal injection per day for 2 weeks. Procedures of MACO model were performed, except that the silicone-coated filament was advanced into the ICA for 5 mm from the common carotid bifurcation without interruption of cerebral blood flow in the middle cerebral artery; (b) sham-operated normal saline (SONS) group: pre-treated with normal saline-water, and subjected to sham operational procedures; (c) MCAO GSPE (MCAOG) group: pretreated with GSPE 50 mg/kg by intra-peritoneal injections per day for 2 weeks, and then subjected to MCAO procedures (d) MCAO normal saline (MCAONS) group: pretreated with normal saline and subjected to MCAO procedures. The neuroprotective effect of GSPE was assessed using behavioral and histological techniques as described below. There were 60 mice in each group. Among the 60 mice, there were 15 for neurological deficit