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Oleuropein, a natural extract from plants, offers neuroprotection in focal cerebral ischemia/reperfusion injury in mice

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ABSTRACT

Oleuropein (OLE) was found to have anti-inflammatory and anti-oxidant effects. The latest study has shown that it can resist myocardial injury that follows an acute myocardial infarction and can rescue impaired spinal nerve cells. In this study, we investigated the neuroprotective effects of OLE on cerebral ischemia and reperfusion injury in a middle cerebral artery occlusion model in mice.OLE (100 mg/kg) was injected intraperitoneally 1 h before ischemia. We found that the volume of cerebral infarction was significantly reduced after 75 min of ischemia and 24 h of reperfusion compared with the I/R (ischemia/reperfusion) group. This protective function occurred in a dose-dependent manner. We also found that treatment with OLE could reduce the cerebral infarct volume. The neuroprotective effect was prolonged from 2 h to 4 h when we injected OLE intracerebroventricularly after reperfusion. We then found that OLE can decrease the level of cleavedcaspase-3, an important marker of apoptosis, in the ischemic mouse brain. Finally, we explored the role of OLE in providing anti-apoptotic effects through the increased expression of Bcl-2 and the decreased expression of Bax, which are important markers in apoptosis. As shown above, the function and safety of OLE in cardiovascular disease may indicate that it is a potential therapeutic for stroke.

1. Introduction

Cerebral infarction is a common cerebrovascular disease throughout the world. Furthermore, its high mortality and disability has resulted in a large social burden (Kikuchi et al., 2014). Although early intravenous thrombolysis therapy is effective in acute cerebral ischemia, many patients fail to receive this treatment because of the time window and contraindications (Virmani et al., 2006). Over the past decade, research has heavily focused on neuroprotection in medicine. Sadly, clinical trials of agents that can provide this neuroprotective effect in animals have not shown the same effectiveness in humans (Auriel and Bornstein, 2010). One of the main reasons for this outcome includes the side effect of these drugs. Therefore, a safe and effective neuroprotective agent is important for the treatment of acute cerebral infarction.

Natural agents from herbs or vegetables have been considered to be ideal and safe materials. Therefore, we have explored useful neuroprotective agents in this area. Oleuropein (OLE), an agent that is naturally obtained from olive leaves, has already been extensively widely used in pharmaceuticals and cosmetics (Barbaro et al., 2014). Previous research has shown that it is safe, as there was no death when researcher used an elevated dose (1 g/kg body weight) of OLE in albino mice. Moreover, its lethal dose could not be determined even while using much larger doses in additional tests (Omagari et al., 2010).

In recent years, OLE's multiple functions have become known in a variety of fields. We already know that OLE has antibacterial activity (Campolo et al., 2013), antiviral activity (Hasegawa et al.,2003), anti-inflammatory activity (Barbaro et al., 2014) and anti-oxidant activity (Garcia-Villalba et al., 2014). Previous studies have also demonstrated that OLE has a potential therapeutic function in different diseases in human. Some studies have shown that OLE could reduce the incidence of coronary atherosclerosis and control blood pressure (Bulotta et al., 2014). OLE also has been explored for its antitumor activity in previous research (Lemonakis et al., 2013). In recent study by Janahmadi and his team, OLE was shown to reduce myocardial injury that is caused by an acute myocardial infarction through the resistance of oxidative stress and anti-inflammatory activity, as anti-inflammation is a major cause of apoptosis (Janahmadi et al., 2015; Ahmad et al., 2014). Other research has indicated that OLE provides neuroprotective effect in spinal cord injury (Khalatbary and Ahmadvand 2012). Because of OLE's lipophilic and small molecular weight,

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Sarbishegi's study indicated that treatment of elderly rats with OLE through oral gavage reduced oxidative damage in the substantianigra pars compacta, by increasing antioxidant enzyme activity (Sarbishegi et al., 2014). We believe that OLE can cross the blood-brain barrier (BBB) when the BBB is broken during cerebral ischemic injury. In this study, we will use an intraperitoneal injection of OLE. Based upon previous research and the pharmacological characteristics of the drug, OLE, a natural agent, may have potential neuroprotective effects against cerebral I/R injury.

In short, we will investigate the neuroprotective function of OLE by using MCAO, which simulates arterial ischemic injury in the brain of mice, to determine related mechanisms.

2. Materials and methods

2.1. Materials

The following reagents were used in the experiment. Oleuropein, 2,3,5-triphenyltetrazoliumchloride (TTC) and mouse monoclonal anti-beta-tubulin were purchased from the Sigma-Aldrich Company (*St* Louis, MO, USA); 6-0monofilaments were purchased from Ethicon Inc. (Somerville, NJ,USA); silicon resin and hardener were purchased from Heraeus Kulzer, LLC. (South Bend, IN, USA); nitrocellulose membrane was purchased from Pall Corp. (Pensacola, FL, USA); ECL chemiluminescence system was purchased from Thermo Company (Rockford, IL, USA); rabbit anti-Bc-2 and rabbit anti-Bax were purchased from Bio-world Technology Inc. (*St* Louis Park, MN, USA); rabbit anti-cleaved-caspase-3 was purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA).

2.2. Animals

Male ICR mice, 23–28 g at two to three months of age, were purchased from the SLAC Company (Shanghai, China). All animal procedures were approved by the University Committee on Animal Care of Soochow University and Yangzhou University.

2.3. Experimental groups

Animals were randomly divided into four groups (n=6 each): (I) vehicle-treated group (sham); (II) OLE-treated group (OLE group); (III) vehicle-treated I/R group (I/R group); or (IV) OLE-treated I/R group (I/R+OLE group). In the I/R+OLE group, the animals were divided into several subgroups (n=6 each): the pre-treatment groups (1 h before ischemia) and the post-treatment groups (different time points). The animals were administered either intraperitoneally (i.p.) at doses of 10, 50 or 100 mg of OLE per kg of body weight or saline or intracerebroventricularly (i.c.v.) at a dose of 10 μg of OLE in 10 ml saline in the OLE-treated groups or 10 μl in the vehicle-treated groups.

2.4. The middle cerebral artery occlusion (MCAO) model

MCAO was induced by using an intraluminal monofilament, as described previously (Xu et al., 2006). Briefly, mice were anesthetized by chloral hydrate and the right common carotid artery (CCA), the right external carotid artery (ECA) and the internal carotid artery (ICA) were exposed through aventral midline neck incision. The ECA was ligated with a silk suture at 2 mm distal from the ECA–CCA branch and then cut distal from the ligated point. A silk suture was looped around the CCA and twisted to block blood flow from the CCA. A small incision was performed on the ECA 1.5 mm distally from the ECA–CCA branch.

A 6–0 nylon monofilament that was coated with siliconresin was introduced through an incision into the right CCA and advanced 9 – 11 mm distal to the carotid bifurcation until a faint resistance was felt. Then, a temporary occlusion of the middle cerebral artery was performed. Reperfusion was achieved by withdrawing the suture after MCAO for the indicated time (30 min or 75 min) in the region whose blood was supplied by MCA. The silk suture that was looped around the CCA was removed and the neck incision was closed. The sham group underwent the same surgical procedure, with the exception of the monofilament being introduced into the external carotid artery, but not being advanced. The body temperature was maintained at 36.5 – 37.5 °C by means of a heating blanket and alamp throughout the procedure (i.e., from the beginning of the surgery until the animals recovered from an aesthesia).

To monitor occlusion and reperfusion, the local cerebral blood flow was measured by a laser-Doppler blood flowmeter (Periflux5010, PERIMED, Sweden) that was positioned at 1 mm posterior and 3 mm lateral to the bregma.

Parameter measurements, including temperature, pH, pO2, pCO2 and plasma glucose, and they measured 30 min before ischemia, 75 min after ischemia and 30 min after reperfusion.

2.5. Intracerebroventricular administration

OLE or saline were injected into the lateral ventricle contralateral to the ischemic side, while a small burr hole was made in the parietal region (0.5 mm posterior and 1.0 mm lateral to the bregma on the left side). A 28 g needle on a syringe was inserted into the left lateral ventricle (2.5 mm in depth). Finally, 10 μl of OLE or saline were injected over a period of 10 min.

2.6. Neurological deficit scoring evaluation

Neurological deficits were evaluated according to the following graded scoring system at 24 h after the MCAO. The modified scoring system was based on a five-point scale system that has been described previously (Wang et al., 2012): 0, no deficit; 1, flexion of the contralateral torso and forelimb; 2, turning to the ipsilateral side when held by the tail; 3, leaning to affected side; or 4, no spontaneous locomotor activity. If no deficits were observed after MCAO, the animal was removed from the study.

2.7. Assessment of the infarct volume

After neurological evaluation, the mice were deeply anesthetized. The brains were removed quickly and frozen (−30 °C) for 2 min immediately. They were then cut into 1-mm slices. Slices were then incubated with 0.2% TTC at 37 °C for 30 min and fixed in 4% paraformaldehyde and then photographed after 24 h of fixation. A computerized image analysis system (Alpha Ease Image Analysis Software V3.1.2; Alpha Innotech Corp., San Leandro, CA, USA) was used to analyze the infarct volume in each brain slice. The percentage of hemispheric infarction volume was calculated as described in our previous study (Yu et al., 2012).

2.8. Western blot analysis

After 75 min of ischemia followed by 24 h of reperfusion, the entire right hemisphere tissue was collected and solubilized by sonication three times for 5 s each and on ice in lysis buffer. The tissue samples were then centrifuged, and the supernatants were used for western-blot analysis. The same amount of total proteins (approximately 30–50 μg) was separated by sodium dodecyl sulfate polyacrylamide gelelectrophoresis and then transferred to nitrocellulose membrane. After blocking with 5% dry milk in phosphate-buffered saline/0.1% Tween 20 (PBST) for 2 h, the blots