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Lab resource

Apigenin attenuates oxidative stress and neuronal apoptosis in early brain injury following subarachnoid hemorrhage

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ABSTRACT

Apigenin (API) is a naturally occurring plant flavone that exhibits powerful antioxidant and antiapoptosis. Oxidative stress plays an important role in the pathogenesis of early brain injury (EBI) following subarachnoid hemorrhage (SAH). The potential anti-oxidative and anti-apoptosis effects of API on EBI following SAH, however, have not been elucidated. The aim of this study was to assess whether API alleviates EBI after SAH via its anti-oxidative and anti-apoptotic effects. The endovascular puncture model was used to induce SAH and all the rats were subsequently sacrificed at 24 h after SAH. Our data demonstrated that administration of API could significantly alleviate EBI (including neurological deficiency, brain edema, blood–brain barrier permeability, and cortical cell apoptosis) after SAH in rats. Meanwhile, API treatment reduced the reactive oxygen species (ROS) level and the concentration of malondialdehyde (MDA) and myeloperoxidase (MPO), elevated the ratio of glutathione (GSH) and oxidized glutathione (GSSG), and increased the amount of super-oxide dismutase (SOD) and hydrogen peroxide in brain cortex at 24 h following SAH. Moreover, API treatment inhibited SAH-induced the expression of Bax and caspase-3, significantly reduced neuronal apoptosis. Collectively, API exerts its neuroprotective effect likely through the dual activities of anti-oxidation and anti-apoptosis, at least partly. These data provide a basic platform to consider API may be safely used as a potential drug for treatment of SAH.

1. Introduction

Apigenin, chemically known as 4,5,7-trihydroxyflavone, with molecular formula $C_{15}H_{10}O_5$, is mainly isolated from the buds and flowers of Hypericum perforatum (Fig. 1) [1,2]. API is found in several dietary plant foods such as parsley, celery, thyme, celeriac, chamomile, onions, lemon balm, and oranges [3]. The potent anti-inflammatory and anti-oxidative activities of API have been extensively reported in various neurodegenerative diseases and epilepsy [4]. Results of Han et al. suggested that API protected against kainite-induced excitotoxicity by quenching ROS as well as decreasing glutathione peroxidase (GSH – Px) depletion [5]. Furthermore, it was reported that API also exhibits neuroprotective effects against oxidative stress-induced cell death by blocking caspase-3 activity in SH-SY5Y cells [6]. API attenuated OGD/R induced neuronal injury mainly by virtue of its anti-apoptosis and anti-oxidative properties in pc12 cells [7].

Subarachnoid hemorrhage (SAH) is a serious condition with high rates of mortality and morbidity in the world [8]. Early in 1990s, early brain injury (EBI) has been recognized to be the primary cause of poor outcomes in SAH patients [9,10]. EBI which occurred during the first 72 h strongly determined the prognosis of the patients suffered SAH [9]. In the complex pathological mechanism of EBI, oxidative stress has been proven to play a crucial role in the process of SAH and may become a novel target for treatment of SAH [11]. Oxidative stress, including lipid peroxidation, occurs following SAH due to excessive free radicals generated by oxyhemoglobin and enzymatic reactions, and plays important role in the pathogenesis of acute brain injury and the breakdown of BBB [12]. Substantial evidences have demonstrated that the level of oxidative stress significantly increases following SAH, and the effects of anti-oxidative therapy in experimental SAH and clinical trials are prominent [13], consequently, the use of free radical scavenger for the treatment of SAH has caused wide attention.

Abbreviations: API, apigenin; EBI, early brain injury; SAH, subarachnoid hemorrhage; ROS, reactive oxygen species; SOD, superoxide dismutase; MDA, malondialdehyde; MPO, myeloperoxidase; GSH, glutathione; GSSG, oxidized glutathione; GSSG, oxidized glutathione; ECA, external carotid artery; ICA, internal carotid artery.

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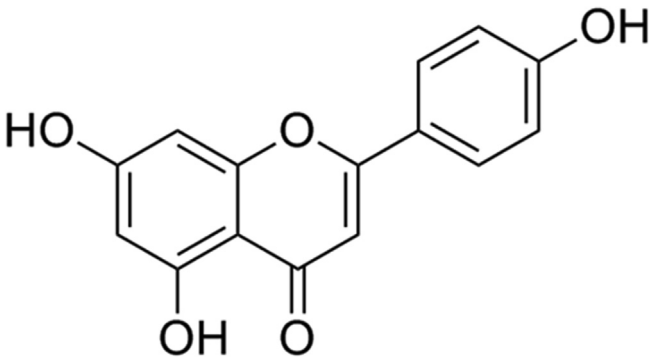

Fig. 1. The chemical structure of the apigenin.

An increasing number of experimental and clinical studies have demonstrated that neuronal apoptosis play pivotal roles in EBI after SAH [14]. Neuronal cells in the brain are highly sensitive to oxidative stress, and neuronal apoptosis is related to the development of neurological deficits [15,16], therefore, the oxidative stress induced by SAH can induce the apoptosis of a large number of neurons, and the process is irreversible. Excessive ROS generation also could induce the damage of neuronal cells. Substantial evidences have revealed there was an intimate relationship between oxidative stress and the development of neuronal death in diverse neurological disorders [17].

Our previous studies demonstrated that API dramatically alleviated EBI following SAH by inhibiting TLR4-mediated inflammatory pathway [18]. Considering oxidative stress plays an important role in the pathogenesis of the EBI after SAH, and API is exactly a potent antioxidant. We speculated that, API may also attenuate EBI of SAH by its anti-oxidative and anti-apoptotic effects.

2. Materials and methods

2.1. Animal preparation and administration

The experimental protocol of using animals was conformed to the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health. Male SD rats were purchased from Experimental Animal Central of General Hospital of Shenyang Military Command (Shenyang, China), fed on standard pellet chow and water *ad libitum*. Rats weighing between 250 g and 300 g were randomly divided into four groups: (1) sham+vehicle group (n = 24), (2) SAH+vehicle group (n = 24), (3) SAH+10 mg/kg API group (SAH+API 10, n = 24), and (4) SAH+20 mg/kg API group (SAH+API 20, n = 24).

API (Tianjin Shilan Technology Co., Ltd, China, 98% pure) was prepared in Tween 80, diluted in 0.9% saline (the final concentration of API was 10 mg/ml and 20 mg/ml and Tween 80 was 10%). API was intraperitoneally (i.p.) administered at 0.5 h after SAH. An equal volume of 10% Tween 80 served as the vehicle control. The dose of API was chosen according to previous study, since beneficial neuroprotective effect was observed [19].

24 h after SAH induction, one quarter of the rats in each group was decapitated for Evans blue (EB) assay, another quarter was decapitated for edema evaluation, the other quarter was given the fixative perfusion for histological preparation and analysis, and the remaining rats were decapitated to obtain tissue samples for the biochemical analysis.

2.2. Experimental SAH rat model

The rat SAH model was performed by endovascular perforation as in previous study [20]. Briefly, rats were anesthetized with 1% sodium pentobarbital (35 mg/kg, i.p.). After exposing the right common carotid artery, external carotid artery (ECA) and internal carotid artery (ICA), the ECA was ligated, cut, and shaped into a 3-mm stump. A sharpened 4–0 monofilament nylon suture was advanced rostrally into the ICA from the ECA stump until resistance was felt (18–20 mm from the common carotid artery bifurcation) and then pushed 3 mm further to perforate the bifurcation of the anterior and middle cerebral arteries. After punctured 15 s, the suture was withdrawn into the ECA stump, and the ICA was reperfused to produce SAH. Sham-operated rats underwent the same procedure without perforation. The incision was then closed, and rats were housed individually following their recovery from anesthesia.

2.3. Neurologic scoring

Neurological scores were blindly evaluated as previously described [21]. The evaluation consisted of 6 tests (spontaneous activity, symmetry in the movement of all 4 limbs, forepaw outstretching, climbing, body proprioception, and response to whisker stimulation) that are scored 0–3 or 1–3. Higher scores indicate greater function.

2.4. Evaluation of the brain edema

After the rats were sacrificed, the whole brain was removed, and then weighted as wet weight. Brain specimens were dried in an oven at 110 °C for 72 h and weighted again (dry weight). The percentage of water was calculated according to the following formula: % H_2O = [(wet weight-dry weight)/wet weigh] × 100.

2.5. Assay of BBB disruption

BBB permeability was quantitatively evaluated by EB extravasation. EB dye (2%, 5 ml/kg; Sigma-Aldrich) was injected into the left femoral vein and allowed to circulate for 1 h. Under deep anesthesia, rats were sacrificed by intracardial perfusion with saline and brains were removed, afterwards, weighed and immersed in formamide (10 ml/g), then incubated at 60 °C for 24 h, the extravasations were measured for absorbance of EB at 620 nm using a spectrophotometer.

2.6. The determination of lipid peroxidation and oxidative stress

The cerebral cortex was obtained and immediately froze in liquid nitrogen until use (n = 6). The activity of Malondialdehyde (MDA), Glutathione (GSH), oxidized glutathione (GSSG) and Superoxide dismutase (SOD) was measured respectively by commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), the catalase activity assay kit was the product of Beyotime Co. (Haimen. China). These experiments were performed according to the manufacturer's instructions. All standards and samples were run in duplicate. The tissue protein concentration was determined using the BCA Protein Assay Kit.

2.7. Myeloperoxidase (MPO) activity

At 24 h after SAH, MPO activity of cortex was evaluated by spectrophotometrically measuring the hydrogen peroxide dependent oxidation of 3,3′,5,5′-tetramethylbenzidine at 460 nm. All procedures were directed by the instruction of MPO kit (Jiancheng Biotechnology, Nanjing, China).