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Cattle Encephalon Glycoside and Ignotin Reduce Early Brain Injury and Cognitive Dysfunction after Subarachnoid Hemorrhage in Rats

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Abstract—Subarachnoid hemorrhage (SAH) is a well-known hemorrhagic stroke with high rates of morbidity and mortality where patients frequently experience cognitive dysfunction. This study explores a potential treatment for cognitive dysfunction following SAH with the demonstration that multi-target drug cattle encephalon glycoside and ignotin (CEGI) can relieve cognitive dysfunction by decreasing hippocampal neuron apoptosis following SAH in rats. Experimentally, 110 male SD rats were separated at random into Sham (20), SAH + Vehicle (30), SAH + 4 ml/kg CEGI (30), and SAH + 1 ml/kg CEGI groups (30) and an endovascular perforation model was created to induce SAH. We discovered that the number of TUNEL-positive neurons in the hippocampus was markedly decreased in SAH + 4 ml/kg and SAH + 1 ml/kg CEGI groups compared to the SAH + Vehicle group. This finding was associated with an observed decrease in Bax/Bcl-2 ratio, cytochrome-c and PUMA expression, and the suppression of caspase-3 activation following SAH. In Morris water maze tests, the SAH + 4 ml/kg CEGI group demonstrated a decreased escape latency time and increase in time spent in the target quadrant as well as crossing times of platform region. These results indicate that high doses of CEGI can decrease hippocampal neuron apoptosis and relieve cognitive dysfunction in rats, suggesting that multitarget-drug CEGI exhibits a neuroprotective effect in SAH via the mitochondrial apoptosis pathway.

Key words: CEGI, subarachnoid hemorrhage, Morris water maze, apoptosis, rats, hippocampus.

INTRODUCTION

Subarachnoid hemorrhage (SAH) is induced by several factors, and carries a high mortality risk as about a quarter of patients with SAH do not survive (Connolly et al., 2012). More than 50% of patients suffer some form of cognitive dysfunction (Kreiter et al., 2002). Approximately 30% of survivors remain permanently handicapped due to neural damage of SAH such as variable neurological dysfunction, including mood disorders, cognitive impairment, and memory damage (Li et al., 2016a,b,c). To date, treatments for these symptoms remain few; because cognitive dysfunction determines the long-term quality of life for SAH survivors, studying effective treatments to protect against cognitive dysfunction following SAH remains essential (see Fig. 1).

Early brain injury (EBI) is a principal cause of mortality and disability in SAH patients (Helbok et al., 2015). Major early brain injuries include blood–brain barrier (BBB) failure, formation of brain edema, and apoptotic cell death of neurons (Park et al., 2004). Apoptosis of neurons occurs primarily in the hippocampus, especially in the CA1 region (Ostrowski et al., 2005; Zhou et al., 2005). The hippocampus and the orbitofrontal cortex (OFC) are key mediators of several cognitive processes that develop cognitive mapping and determine behavior as demonstrated in the cognitive map method of hippocampal function (Wikenheiser and Schoenbaum, 2016). The hippocampus is important for spatial representation of the environment and memory of specific events. Specifically, this part of the brain has four major computational functions: (i) helping to bridge shifts in context, (ii) connecting internal and external cues, (iii) creating search procedures for planning, deliberation, and identification, and (iv) providing a resource for consolidation (Lisman et al., 2017). Finally, apoptosis of neurons in the hippocampus can lead to severe cognitive dysfunction (Li et al., 2010).

The cattle encephalon glycoside and ignotin (CEGI) injection was approved to treat Alzheimer's disease by the Chinese Food and Drug Administration in 2011 (Formulation: 3.2 mg/ml polypeptides, 0.24 mg/ml monosialotetrahexosyl ganglioside (GM-1), 1.65 mg/ml

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Abbreviations: BBB, blood–brain barrier; CEGI, cattle encephalon glycoside and ignotin; CPP, cerebral perfusion pressure; EBI, early brain injury; ECA, external carotid artery; GM-1, monosialotetrahexosyl ganglioside; ICA, internal carotid artery; ICP, intracranial pressure; MOMP, mitochondrial outer membrane permeabilization; MWM, Morris water maze; SAH, subarachnoid hemorrhage; SCI, spinal cord injury; TUNEL, TdT-mediated dUTP Nick-End Labeling.

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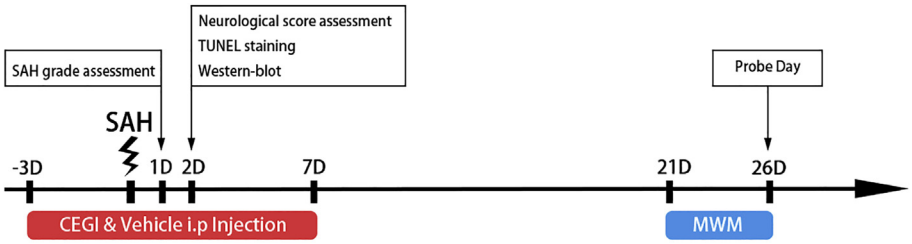

Fig. 1. Flowchart to visualize the experiment procedure and time point.

free amino acids, 0.925 mg/ml total nitrogen, and 12 μg/ml hypoxanthine; drug approval H22025046; Jilin Sihuan Pharmaceutical Co. Ltd., Jilin, People's Republic of China). CEGI has been shown to reduce white matter injuries and prevent post-hemorrhagic hydrocephalus in a intracerebral hemorrhage rat model (Li et al., 2016a,b,c). It has also been shown to improve diabetic peripheral neuropathy and treat neonatal hypoxic ischemic encephalopathy; however, to our knowledge, no study has investigated if CEGI eventually leads to an improvement in neurological outcomes after experimental SAH.

GM-1 is a widely utilized component for treating spinal cord injury (SCI). The Sygen Clinical trials reported that GM-1 had a significant therapeutic effect on SCI patients. When compared with GM-1 and placebo, the SCI Frankel grade of patients increases by more than two grades between the start of the study and at 1-year of follow up (Geisler, 1998). The acute neuroprotective and long-term regenerative effects of GM-1 in experimental models of ischemia and injury have been well demonstrated (Ramirez et al., 1987; Skaper and Leon, 1992; Wang et al., 1995; Lainetti et al., 1998; Itoh et al., 2000; Sautter et al., 2000). Additionally carnosine, commonly thought to be the active component of CEGI, has several effects on mitochondrial protection and the attenuation of deleterious autophagic processes in both rat focal ischemia and neuronal cultures (Baek et al., 2014). Furthermore, carnosine has been demonstrated to protect rats against hypoxia–ischemia-induced brain damage through antioxidation (Zhang et al., 2011).

In the present study, apoptosis of neurons in CA1 region caused by SAH EBI was demonstrated to lead to cognitive dysfunction (Kadar et al., 1998). Therefore, an intravascular puncture SAH rat model was designed to demonstrate the effects of CEGI on neurological function and apoptosis of neurons in the hippocampus after SAH in rats. This study also aimed to assess the protective properties and mechanism of CEGI in relation to SAH by investigating an innovative technique for treating cognitive dysfunction following SAH.

EXPERIMENTAL PROCEDURES

Animals and experimental groups

According to the guidelines of the China Food and Drug Administration, CEGI is usually administered at a dosage of 5–20 ml/60 kg via intravenous injection for adult patients. We used the Meeh–Rubner formula to calculate the body surface area of humans and rats for estimation of the proportional drug dose (Ohwada, 1992). According to the formula, the body surface area of a 60 kg person is about 1.55 square meters, and a 250–300 g rat is about 0.036–0.041 square meters. According to this body surface area conversion, the minimum drug dosage is 0.12–0.13 ml per rat, the conversion unit is 0.43–0.48 ml/kg. Therefore, in order to select a simple and easy-to-manipulate method of administration, we used intraperitoneal injection and increased the dosage. Specifically, 1 ml/kg/d CEGI was adopted in our experiments as the minimum dosage, 4 ml/kg/d CEGI as the maximum dose according to human dosage range.

In this study, 147 adult male Sprague–Dawley rats weighing between 250 and 300 g were used. 28 rats were used for SAH grade assessment to evaluate the severity of SAH on the basis of subarachnoid blood between groups. Among them, four rats due to low SAH grade (SAH grades $\leq$7 at 24 h post-SAH; SAH + vehicle, n = 2; SAH + 1 ml/kg/d CEGI, n = 1; SAH + 1 ml/kg/d CEGI, n = 1) were excluded. These rats were sacrificed and brain tissue was used for scoring and could not be used for subsequent experiments or for mortality assessment. The remaining 119 rats were randomly divided into five treatment groups: sham (n = 20), SAH + vehicle (4 ml/kg/d; n = 34), SAH + CEGI (1 ml/kg/d; n = 33), SAH + CEGI (4 ml/kg/d; n = 32), nine rats got 0 point in neurological function assessment at 48 h post-SAH were excluded for subsequent experiments or for mortality assessment (SAH + vehicle, n = 4; SAH + 1 ml/kg/d CEGI, n = 2; SAH + 1 ml/kg/d CEGI, n = 3). Drugs and vehicle (0.9% NaCl) were administered by i.p (intraperitoneal) injection.

SAH models

An endovascular perforation model was performed to produce SAH (Chen et al., 2013). Rats were anesthetized with an intraperitoneal injection of chloral hydrate (400 mg/kg). A heating pad was utilized to preserve rectal temperature at 37 °C during operation. The external carotid artery (ECA) was ligated and fashioned into a stump. A sharpened 4-0 monofilament nylon suture was advanced into the internal carotid artery (ICA) from the ECA stump through the common carotid bifurcation. The suture was further advanced into the intracranial ICA until resistance was felt and then pushed 3 mm further to perforate the ICA wall. After puncturing, the suture was removed, and the ICA was opened for reperfusion to produce SAH. Sham rats were exposed to the same procedure without vessel perforation. All animals had free access to food and water.

Neurological score assessment

Neurobehavioral deficits were blindly assessed with three behavioral activity examinations using the scoring system