Brain Research Bulletin 134 (2017) 24–29

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

The effect of monascin on hematoma clearance and edema after intracerebral hemorrhage in rats

Juan Wang[a], Gaiqing Wang[a,b,*], Jinying Yi[a], Yi Xu[c], Shuna Duan[a], Tong Li[a], Xin-gang Sun[b], Liang Dong[a]

[a] Department of Neurology, Shanxi Medical University, 56 Xinjian S Rd, Yingze, Taiyuan, Shanxi, 030001, China
[b] Department of Neurology, The Second Hospital, Shanxi Medical University, 382 WuYi Avenue, Taiyuan, Shanxi, 030001, China
[c] Department of Radiology, The Second Hospital, Shanxi Medical University, 382 WuYi Avenue, Taiyuan, Shanxi, 030001, China

ARTICLE INFO

Keywords:
Intracerebral hemorrhage
Monascin
Hematoma clearance
Neuroprotection
Magnetic resonance imaging

ABSTRACT

Background and purpose: Intracerebral hemorrhage (ICH) is a particularly devastating form of stroke with high mortality and morbidity. Hematomas are the primary cause of neurologic deficits associated with ICH. The products of hematoma are recognized as neurotoxins and the main contributors to edema formation and tissue damage after ICH. Finding a means to efficiently promote absorption of hematoma is a novel clinical challenge for ICH. Peroxisome proliferator-activated receptor gamma (PPARγ) and nuclear factor erythroid 2-related factor 2 (Nrf2), had been shown that, can take potential roles in the endogenous hematoma clearance. However, monascin, a novel natural Nrf2 activator with PPARγ agonist, has not been reported to play a role in ICH. This study was designed to evaluate the effect of monascin on neurological deficits, hematoma clearance and edema extinction in a model of ICH in rats.

Methods: 164 adult male Sprague-Dawley (SD) rats were randomly divided into sham; vehicle; monascin groups with low dosages (1 mg/kg/day), middle dosages (5 mg/kg/day) and high dosages (10 mg/kg/day) respectively. Animals were euthanized at 1, 3 and 7 days following neurological evaluation after surgery. We examined the effect of monascin on the brain water contents, blood brain barrier (BBB) permeability and hemoglobin levels, meanwhile reassessed the volume of hematoma and edema around the hematoma by Magnetic Resonance Imaging (MRI) in each group.

Results: The high dosage of monascin significantly improved neurological deficits, reduced the volume of hematoma in 1–7 days after ICH, decreased BBB permeability and edema formation in 1–3 days following ICH.

Conclusion: Our study demonstrated that the high dosage of monascin played a neuroprotective role in ICH through reducing BBB permeability, edema and hematoma volume.

1. Introduction

ICH accounts for 8–15% of all strokes in Western societies and 20–30% among Asian populations, and most patients either die or are left with significant neurological deficit (Keep et al., 2012; Sangha and Gonzales, 2011). Clinical treatment of ICH presently consists of decompressive surgery in selected cases and supportive measures to reduce bleeding and control hypertension (Brouwers and Goldstein, 2012; Fischer et al., 2016). Given the enormity of the clinical problem, it is imperative that new therapeutic approaches should be developed to improve outcome following ICH.

During past years, the transcription factors of PPARγ (Aronowski and Zhao, 2011; Zhao et al., 2009) and Nrf2 (Zhao et al., 2015a,b) were received as important players in regulating phagocyte-mediated cleanup processes. PPARγ and Nrf2 play an important role in phagocytosis and hematoma clearance after ICH (Zhao et al., 2015a,b). Monascin- a major component of red yeast rice, acts as a PPARγ agonist, were confirmed to regulate the expression of Nrf2 (Hsu et al., 2013, 2014; Lee et al., 2011). Effects of monascin after ICH have not been evaluated yet and the findings, as mentioned above, let us hypothesize that monascin will have beneficial, clinically translatable effects after ICH.

In this study, we suggest that monascin will promote hematoma clearance via PPARγ and Nrf2 up-regulation, therefore reducing brain edema and improving BBB integrity after ICH in a collagenase infusion model in rats.

* Corresponding author at: Department of Neurology, The Second Hospital, Shanxi Medical University, 382 WuYi Avenue, Taiyuan, Shanxi, 030001, China
E-mail addresses: wanggq08@163.com, wanggq08@126.com (G. Wang).

http://dx.doi.org/10.1016/j.brainresbull.2017.06.018
Received 17 March 2017; Received in revised form 24 May 2017; Accepted 21 June 2017
Available online 24 June 2017
0361-9230/

2. Material and methods

2.1. Animals

Twelve-week-old male SD rats (weight 275g ± 25 g, Animal Experimental Center of Shanxi Medical University) were used in this study. All experimental procedures were conducted in accordance with the Care and Use of Laboratory and approved by the Committee of Shanxi Medical University. Rats were given free access to food and water throughout the study.

2.2. Animal treatments and experimental groups

A total of 164 rats were used. Animals were randomly divided into the following groups: sham (n = 30); Vehicle (n = 34); ICH + low dosage monascin (1 mg/kg/day, n = 34); ICH + middle dosage monascin (5 mg/kg/day, n = 33); ICH + high dosage monascin (10 mg/kg/day, n = 33). Each group was equally divided into three subgroups (point at 1, 3, 7 days after ICH). Animals, which died before final assessment, were replaced. All gavages were administered intragastrically 6 h after ICH and twice a day until the euthanasia point.

2.3. Intracerebral hemorrhage model in rats

Experimental ICH was induced by stereotactic-guided injection of collagenase type IV (0.5 units in 2 μl saline) into the basal ganglia areaas we previously described (Wang et al., 2016). The rats were fastened on a stereotaxic apparatus under chloral hydrate anesthesia (0.8 mg/kg intraperitoneally), exposed the skull and reveal bregma. A 1-mm cranial bur hole was drilled in the skull (coordinates: 1.0 mm posterior to the bregma, 3.0 mm lateral to the midline), and microinjector was inserted with collagenase infused into the right basal ganglia (5.8 mm deep from the dura mater). The needle was left in place for an additional 10 min after injection after injection to prevent "back-leakage" and then slowly withdrawn over 5 min. The Sham-operated rats were syringed with equivalent dosages physiological saline. After the surgery, the skull hole was sealed with bone wax and the incision was closed with sutures. Animals were allowed to recover after successful ICH induction that was confirmed by Rosenberg's neurological score (Rosenberg et al., 1990).

2.4. Analysis of neurological deficit score

All behavioral tests were conducted in a quiet and low light room by an independent researcher blinded to the procedure by the Garcia test (Garcia et al., 1995; Wang et al., 2017). Neurological symptoms were calculated by combining the score as follows (score: 2–18): (1) spontaneous activity, (2) symmetry in the movement of four limbs, (3) forepaw outstretching, (4) mesh wall climbing, (5) body proprioception, (6) response to vibrissae touch. Higher scores indicate greater neurofunction (healthy rat).

2.5. Brain water content

Animals were euthanized under deep anesthesia and decapitated for brain water content determination as previously described (Wang et al., 2017). The brains were quickly removed and cut into 4 mm sections around the puncture point. All specimens obtained from ipsilateral basal ganglion were immediately weighed on an analytical microbalance to obtain the wet weight. The tissue was then dried at 100 °C for 48 h to determine the dry weight. Brain water content was calculated as percentage of (Wet weight-Dry weight)/Wet weight × 100.

2.6. The blood brain barrier (BBB) permeability measurement

The vascular permeability of BBB was evaluated with Evans blue (EB) extravasation method (Manaenko et al., 2011). Three hours before each experiment, 2% EB (4 ml/Kg) was injected into the abdominal cavity. After the circulation period, the rats were perfused with 100 ml of ice-cold phosphate-buffered saline (PBS). The brain tissue was quickly removed. The samples were homogenized in 1100 μl of PBS, sonicated and centrifuged (30 min, 15,000 rcf, 4 °C). The supernatant was collected in aliquots. For each 500 μl sample an equal amount of 50% trichloroacetic acid was added, incubated over night by 4 °C and then centrifuged. Optical density was measured and recorded at 540 nm with a spectrophotometer. The dye content was expressed as ug/g of tissue weight and calibrated with a standard curve obtained from known amounts of the EB dye. The data was represented as a ratio compared to sham.

2.7. Hemoglobin assay

The supernatant collection was completed as the BBB permeability measurement. Following the supernatant collection, 100 μl supernatant aliquots was added to 400 μl Drabkin's reagent. The hemoglobin assay was measured by spectrophotometer at 540 nm.

2.8. Western blot

Western Blot was performed for proteins as previously described (Wang et al., 2017). Briefly, the right cerebral hemispheres were homogenized, and protein concentration acquired for each sample using a detergent compatible assay (Bio-Rad, Philadelphia, PA). Protein samples (50 μg) were loaded on a tris–glycine gel, electrophoresed, and transferred to a nitrocellulose membrane. Membranes were incubated overnight at 4 °C with the primary antibodies: rabbit polyclonal anti-PPARγ (1:750, abcam), anti-Nrf2 (1:1000, abcam). The same membrane was probed with an antibody against β-actin (1:3000, bioworld) as an internal control after being stripped. Incubation with secondary antibodies (bioworld) was done for 2 h at room temperature. Immunoblots were then probed with an ECL Plus chemiluminescence reagent kit (Amersham Biosciences, Arlington Heights, IL) and visualized with an imaging system (Bio-Rad, Versa Doc, model 4000). Data was analyzed using Alpha View software.

2.9. Assessments of hematoma volume and brain edema

The cranial MRI examinations were obtained on a 1.5T clinical scanner (GE SignaHDx, GE healthcare Milwaukee). A knee coil was used for radio frequency transmission and reception. Studies were performed at 1, 3, 7 days post-ICH. During the MRI experiments, anesthesia was maintained using 5% chloral hydrate, and placed in a prone position. After set-up, a series of MR images, including T2-weighted imaging (T2WI), T2 fluid attenuated inversion recovery (T2 Flair), T2*-weighted imaging (T2*WI), and susceptibility-weighted imaging (SWI), were acquired.

The scan sequence and details were listed as follows: (1) T2WI: repetition time (TR)/echo time (TE) = 2400/129.2 ms, matrix was = 512 × 448, field of view (FOV) = 18 × 18 mm, slice thickness = 2.0 mm, interval = 0.2 mm. (2) T2 Flair: TR/TE = 8502/128.6 ms, matrix was = 512 × 448, FOV 12 × 12 mm, slice thickness = 2.0 mm, interval = 0.2 mm. (3) T2*WI: TR/TE = 400/15 ms, matrix was = 448 × 384, FOV = 18 × 18 mm, slice thickness = 2.0 mm, interval = 0.2 mm, flip angle = 15°. (4) SWI: TR/TE = 49.9/4.5 ms, matrix was = 448 × 448, FOV = 18 × 18 mm, slice thickness = 1.5 mm, flip angle = 15°. Hematoma size was determined from T2*WI and SWI, T2WI and T2 Flair were used for edema quantification. The measurements were performed by an examiner blinded to the exam date using a computer-assisted image analysis program-Image J (Jack, 1994). The volumes were calculated by summation of lesion areas of all brain slices showing brain damage and integrated by slice thickness. The absolute volume of brain