Int J Clin Exp Med 2017;10(9):13100-13108
www.ijcem.com /ISSN:1940-5901/IJCEM0049058

Original Article

Effects of Huanglian-Jie-Du-Tang extract on global cerebral ischemia injury *via* anti-inflammation reaction in mice

Yi-Lu Ye[1], Li-Li Jiang[2], Zhu-Yan Huang[3], Bei-Bei Pan[3], Jing Xu[1,4], Kai Zhong[1], Yue-Ping Yu[1], Qi Zhang[1]

[1]Department of Pharmacology, Hangzhou Medical College, Hangzhou, China; [2]Zhejiang Hospital, Hangzhou, China; [3]The Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, China; [4]Wenzhou Medical University, Wenzhou, China

Received January 17, 2017; Accepted June 28, 2017; Epub September 15, 2017; Published September 30, 2017

Abstract: The Chinese herbal formula Huanglian-Jie-Du-Tang (HJDT) is composed of the roots of *Coptis chinensis Franch.*, *Scutellaria baicalensis Georgi.*, *Phellodendron chinense Schneid.*, the fruit of *Gardenia jasminoides Ellis*, and is widely used in clinical. This study investigates the neuroprotective effects of HJDT extract on global cerebral ischemia (GCI) in mice. GCI was induced by occluding bilateral common carotid arteries for 30 min. HJDT (4 g/kg) and minocyline were administered orally and intraperitoneally from day 7 before until day 7 after ischemia, respectively. The behavioral tests were conducted 1 h after treatment at day 7 after GCI. The Western-Blot and ELISA methods were used to evaluate the expression of Iba-1, and the contents of TNF-α and IL-1β, respectively. HJDT had no effect on the total traveled distance, but decreased the central time during 15-30 min in locomotor activity test. In addition, HJDT significantly increased the discrimination ratio (DR) and discrimination index (DI) in NOR test, improved the morphological change of neurons, and increased the neuron density, especially in the hippocampal CA1 region. Also HJDT decreased the expression of Iba-1, the contents of IL-1β, and TNF-α at day 7 after ischemia in the hippocampal CA1 region. These results suggested that HJDT exerted neuroprotective effects on GCI *via* inhibiting microglia activation and decreasing the release of inflammatory cytokines.

Keywords: Huanglian-Jie-Du-Tang (HJDT), global cerebral ischemia (GCI), anti-inflammation reaction, mice

Introduction

Cerebral ischemia is a common clinical cerebrovascular disease. Restoring blood flow, called reperfusion, within 30 min after transient cerebral ischemia is vital to reduce widespread neuronal necrosis and improve functional recovery of tissue [1]. However, reperfusion may also exacerbate brain injuries, a condition called cerebral ischemia/reperfusion (I/R) injury [2]. The mechanism of I/R injury is complicated. One of the pathophysiologies of cerebral ischemia includes the actions of different cell types as well as a large number of inflammatory cytokines [3]. For example, early in ischemic injury, microglia are activated in response to cerebral ischemia injury, which synthesize and release harmful inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), reactive oxygen species, and so on [4, 5]. This reaction ultimately cause delayed neuronal damage in addition to cerebral injury [6], although the role of microglia is still being debated.

Huanglian-Jie-Du-Tang (HJDT) is a traditional Chinese herbal formula, composed of four herbs: the roots of *Coptis chinensis Franch.*, *Scutellaria baicalensis Georgi.*, *Phellodendron chinense Schneid.*, and the fruit of *Gardenia jasminoides Ellis*. HJDT is widely used for alleviating the symptoms of gastrointestinal issues [7], liver injury [8], Alzheimer's diease [9], ischemic brain injury [10-12], and so forth. A previous study, conducted by this group, has found HJDT had protective effects against focal cerebral ischemia [13].

Both global cerebral ischemia (GCI) and focal cerebral ischemia animal models were used to investigate the neuroprotective effects of these agents. Compared to focal cerebral ischemia,

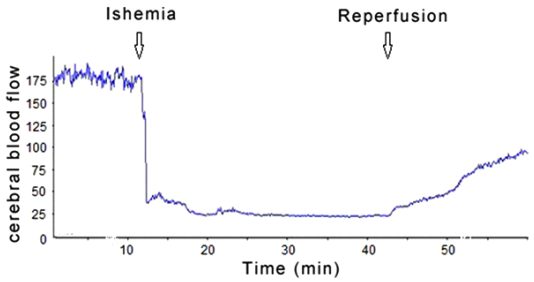

Figure 1. Change of cerebral blood flow of GCI for 30 min following reperfusion.

GCI was the model that most closely resembles clinical situations such as cardiac arrest, near drowning, or severe systemic hypotension during surgical procedures [14, 15]. As such, this model was considered a relevant and reproducible rodent model of cerebral ischemia.

Whether HJDT had neuroprotective effects on GCI has not been reported yet. Thus, this study investigated the effects of HJDT on GCI with a series of behavioral tests and further explored the effects of HJDT on microglia and inflammation cytokines.

Materials and methods

Animals

Male C57BL/6 mice, weighing 23-25 g, were purchased from Shanghai Slyke Laboratory Animal Limited Corporation (Certificate No. SCXK 2012-0002, Shanghai, China) and were housed socially in cages under a controlled temperature (22 ± 2°C) with a 12-h day/night cycle. The animals were allowed free access to food and water and were left to acclimate for 7 days, during which time they were handled repeatedly before test. All experiments were carried out in accordance to Guide for the Care and Use of Laboratory Animals provided by the National Institute of Health.

Preparation of HJDT

HJDT is consisted of the roots of *Coptis chinensis Franch.*, *Scutellaria baicalensis Georgi.*, *Phellodendron chinense Schneid.*, and the fruit of *Gardenia jasminoides Ellis*, at a ratio of 3:2:2:3, which were purchased from Eastern China Medical Corp., Zhejiang Province, China. Raw components of the HJDT formulation were mixed and soaked in distilled water for 1 h. Then they were boiled for 2 h in distilled water, 1.5 h in 50% ethanol, and then 1.5 h in 80% ethanol. At last, all the filtrates were mixed and concentrated in a rotary evaporator under reduced pressure to a concentration of 1.0 g/ml.

Drugs and treatments

The mice were randomly divided into four groups: sham group, GCI group, HJDT (4 g/kg) group, and minocyline (MINO) (45 mg/kg) group. Each group consisted of 12 mice. HJDT was administered orally while minocycline was intraperitoneally injected to their respective groups from day 7 before until day 7 after ischemia. The control and GCI mice were administered saline orally. Minocyline was obtained from the Sigma Corporation of America.

GCI model

GCI was induced as previously described by Murakami K. [16]. Briefly, bilateral common carotid arteries were isolated and the blood flow was occluded by arterial clamps for 30 min. After 30 min, the clamps were released to restore arterial blood flow in the brain. The change of cerebral blood flow was monitored in real-time with a Laser Doppler Instrument (J&K Chemical LTD, Sweden) during the course of the operation. Cerebral blood flow decreased to less than 20% and was considered as a successful GCI model (**Figure 1**). The bilateral common carotid arteries of the sham group were only exposed and isolated, but did not have their cerebral blood flow blocked.

Locomotor activity test

Locomotor activity was measured and analyzed using a Spontaneous Activity Video Analysis System (JLBehv-LAG-4, Shanghai Jiliang Software Technology Co., Ltd. China). The size of the square enclosure was (40 cm × 40 cm × 60 cm) and was divided into a central region (20 cm × 20 cm) and a peripheral region. Each mouse was placed gently into the center of the enclosure 1 h after administration of the drugs. The locomotor tracks were continuously recorded by video camera for 1 h and analyzed. The following parameters: (a) total traveled distance in the central and peripheral regions, as well as (b) time in central and peripheral regions were calculated. After each test session, the enclo-

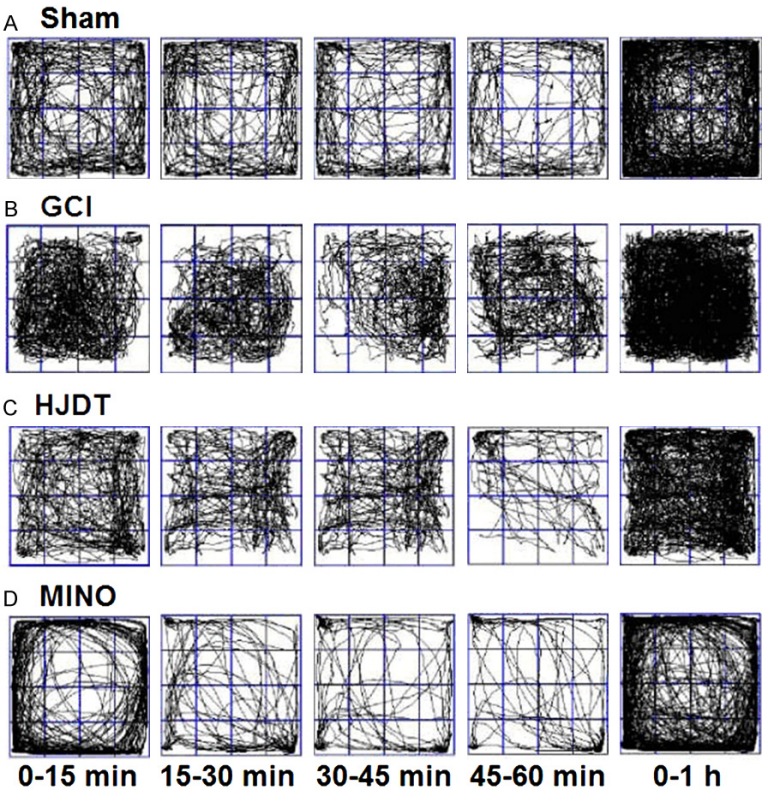

Figure 2. The typical locomotion tracks at 15 min intervals and within 1 h after administration in mice.

sures were thoroughly cleaned with a 70% ethanol solution and water.

Novel object recognition (NOR) test

NOR test consists of 3 phases: T_1 phase, interval time, and T_2 phase. In the T_1 phase, two identical objects (familiar object) were put in two opposite corners of the open field. The mice were individually placed in the open field to explore for 10 min and then removed back into their home cages. After a 1.5 h interval time, the mice were put into the open field again. T_2 phase started with one of the familiar objects replaced by a new object (novel object). The mice then were allowed to explore for 5 min. Tracks were recorded and analyzed by a computerized video analyzer (EthoVision XT, Noldus, Netherlands). The following parameters were analyzed: discrimination ratio (DR, DR = [N/(N + F)] × 100%) and discrimination index (DI, DI = [(N - F)/(N + F)] × 100%). (N: the time to explore a novel object, F: the time to explore familiar object). Exploration was defined as the distance between the mouse nose and the object ≤ 2 cm when it faces the object.

Histopathological examination

The mice were anesthetized after the neurological evaluation on day 7 after GCI and were perfused transcardially with 4% paraformaldehyde after pre-flushing with ice-cold saline. Brains were removed, fixed with the same fixative for 24 h, and then stored in 30% sucrose solution at 4°C for 1-2 days. The brains were cut into 8 μm coronal sections on a cryomicrotome (CM1900, Leica, Germany) -2.0 mm from bregma. The 8 μm sections were used for neuron density in the hippocampal CA1 region, temporal cortex and striatum by cresyl violet dyeing. The number of healthy-looking neurons (large cells with a pyramidal shape) was counted in sections.

Western-blot method

Samples of hippocampus were homogenized on ice in PBS containing 1% protease inhibitor and 1% phosphatase inhibitor for Western-blot. Proteins were obtained by centrifugation at 14000 rpm at 4°C for 15 min and quantified by Bradford assay (BioRad, USA). A 50 μg sample of each group was subjected to electrophoresis using 20% SDS at 80 V. The proteins were transferred to polyvinylidene fluoride membranes at 250 mA for 2 h. Antibodies for Iba-1 (1:1000, Wako, Japan) and GAPDH (1:10000, KangChen Bio-tech, Shanghai, China) were applied overnight. Then membranes were incubated with horseradish peroxidase (HRP) secondary antibody (KangChen Bio-tech, Shanghai, Chima). At last, the signal intensities of proteins were analyzed using Image J software.

ELISA method

Mice were decapitated and the hippocampus was separated on ice quickly at day 7 after GCI. Then it was sonicated to obtain tissue homogenates. After removing particulars by centrifugation (2000×g, 4°C, 20 min), assay was immediately detected. IL-1β and TNF-α were

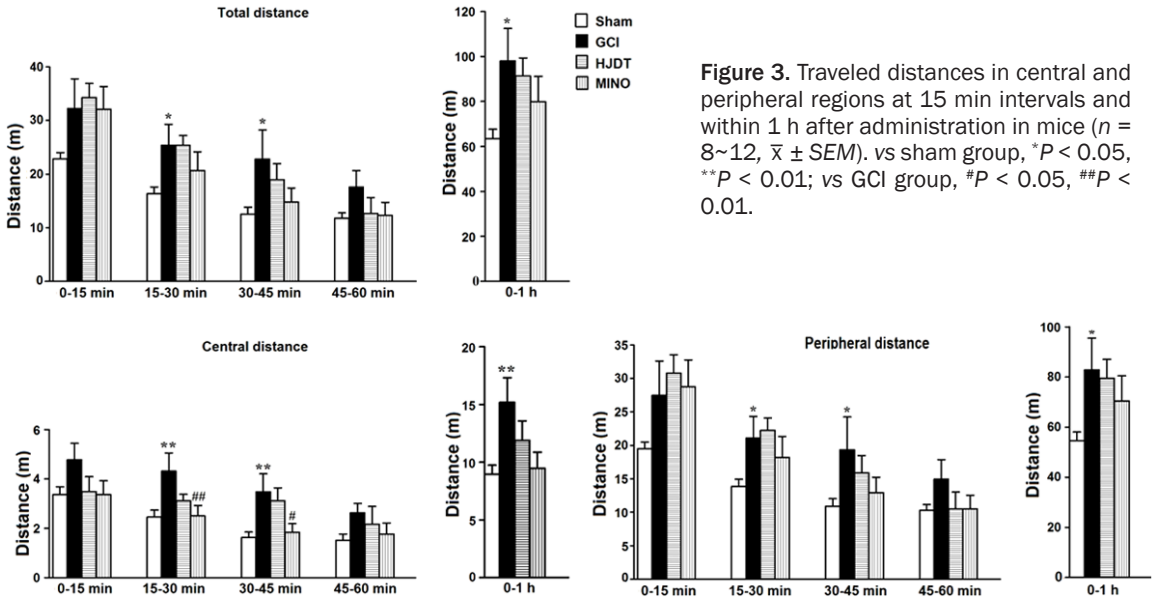

Figure 3. Traveled distances in central and peripheral regions at 15 min intervals and within 1 h after administration in mice (n = 8~12, $\bar{x} \pm SEM$). *vs* sham group, $^{*}P < 0.05$, $^{**}P < 0.01$; *vs* GCI group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.

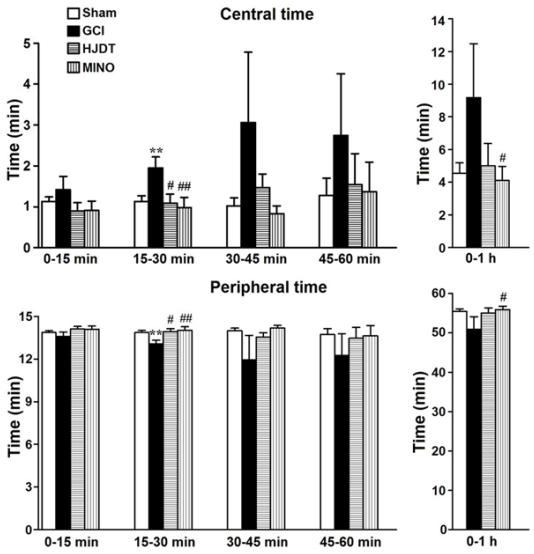

Figure 4. Spent time in central and peripheral regions at 15 min intervals and within 1 h after administration in mice (n = 8~12, $\bar{x} \pm SEM$). *vs* sham group, $^{**}P < 0.01$; *vs* GCI group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.

measured using respective ELISA system (Shanghai Elisa Biotech Co., Ltd, China) according to the manufacturer's instructions. Finally, optical density was determined (absorbance at 450 nm) on a plate reader.

Statistical analysis

The data were expressed as mean ± standard error (SEM). Statistical comparisons were performed using one-way analysis of variance (ANOVA) following with Fisher's least significant differences (LSD) post hoc analysis tests. A $P < 0.05$ was considered statistically significant.

Results

Effects of HJDT on locomotor activity test

HJDT improved the regioselectivity in locomotor activity: During the locomotor activity test, the sham group explored the new environment actively, and demonstrated the most activity in the first 15 min. Furthermore, the sham mice showed significant regioselectivity. They spent more time and traveled more distance in the peripheral region. Overall, the activity amount increased after GCI, but the regioselectivity was lessened. HJDT (4 g/kg) and minocyline decreased the overall traveled distance while improving the regioselectivity (**Figure 2**).

HJDT had no effect on traveled distance in locomotor activity test: There was no significant difference in traveled distance during 0-15 min and 45-60 min among groups. After GCI, the total distance, central distance, and peripheral distance increased during 15-30 min, 30-45 min, and 0-1 h. Minocyline decreased the central distance during 15-30 min and 30-45 min. There was no significant effect on traveled distance after treated with HJDT (**Figure 3**).

HJDT decreased the central time during the 15-30 min in locomotor activity test: During 15-30 min, the sham group spent (1.13 ± 0.14)

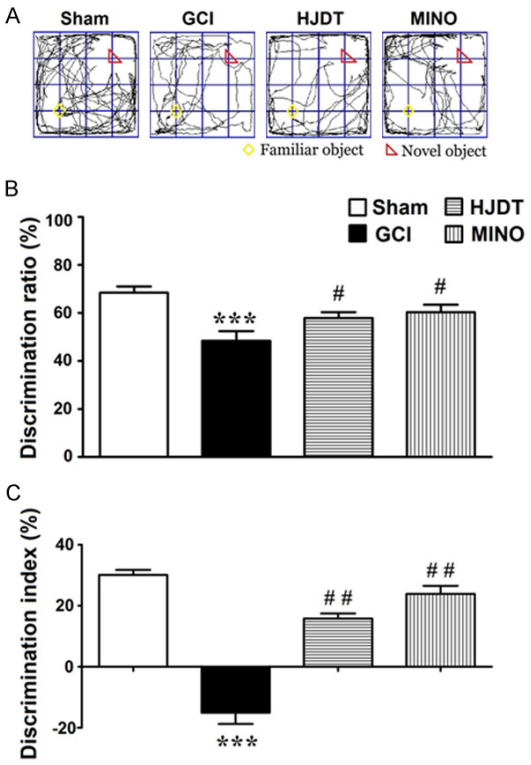

Figure 5. The typical tracks of mice in T2 phase in NOR test (A) and the effect of HJDT on the novel object DR (B) and DI (C) in mice (n = 8~12, $\bar{x} \pm SEM$). *vs* sham group, $^{***}P < 0.001$; *vs* GCI group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.

min in the central region and (13.87 ± 0.14) min in the peripheral region. However, in GCI group, the central time increased to (1.95 ± 0.27) min and peripheral time decreased to (13.05 ± 0.27) min during the 15-30 min duration in the test. Minocyline and HJDT (4 g/kg) reversed this effect during the 15-30 min section. Also, minocyline decreased the central time and increased the peripheral time within 1 h, but HJDT had no effect within 1 h after administration in mice (**Figure 4**).

HJDT increased DR and DI in NOR test

Performance in the NOR test for hippocampal-dependent memory was tested in this section. The sham group spent more time to explore the novel object than the familiar in mice. DR was (68.4 ± 2.6)% and DI was (29.5 ± 6.2)% in the control group. GCI significantly affected novel object recognition, as DR and DI for exploring novel object decreased to (48.4 ± 4.0)% and (-15.0 ± 10.5)%, respectively. HJDT (4 g/kg) and minocyline groups significantly increased DR and DI, indicating that they recognized the novel object as the new (**Figure 5**).

HJDT increased the neuron density after GCI

In the hippocampal CA1 region, cortex, and striatum, the morphology of the neurons observed were obviously withered and the density of the apparently surviving neurons had almost disappeared at day 7 after GCI (**Figure 6**). HJDT (4 g/kg) and minocyline improved the morphological change of neurons and increased the neuron density, especially in the hippocampal CA1 region.

HJDT decreased the expression of Iba-1, IL-1β and TNF-α after GCI in the hippocampus

Compared with the sham group, the expression of Iba-1, as well as the contents of IL-1β and TNF-α both increased, at day 7 after GCI in the ischemic periphery. The results of WB showed fewer expression of Iba-1 in the hippocampus. But it significantly increased after GCI. Treatment with minocyline or HJDT reduced the expression of Iba-1 (**Figure 7**). The results of ELISA showed the contents of IL-1β and TNF-α increased at day 7 after GCI. HJDT treatment decreased the contents of IL-1β and TNF-α by 43.79% and 27.74% after GCI, respectively. Minocyline showed similar effects with HJDT (**Figure 8**).

Discussion

In this study, it was demonstrated that HJDT clearly improved the regioselectivity in locomotor activity test, learning-memory ability, and pathological change of neurons in the hippocampal CA1 region after GCI. In addition, HJDT decreased the expression of Iba-1, TNF-α and IL-1β in the hippocampus.

Spontaneous locomotor activity is often evaluated using the locomotor activity test and is one of the most basic behavioral tests used in psychological and pharmacological investigations (usually in rodents). The spatial and temporal organization are the most important properties of locomotor activity test in addition to the amount of activity exhibited by rodents [17]. Milot. reported [18] the total activity distance, central distance ratio, and central time ratio increased at day 5 after GCI. Our study also found the same results at day 7 after GCI. HJDT (4 g/kg) significantly increased the central time

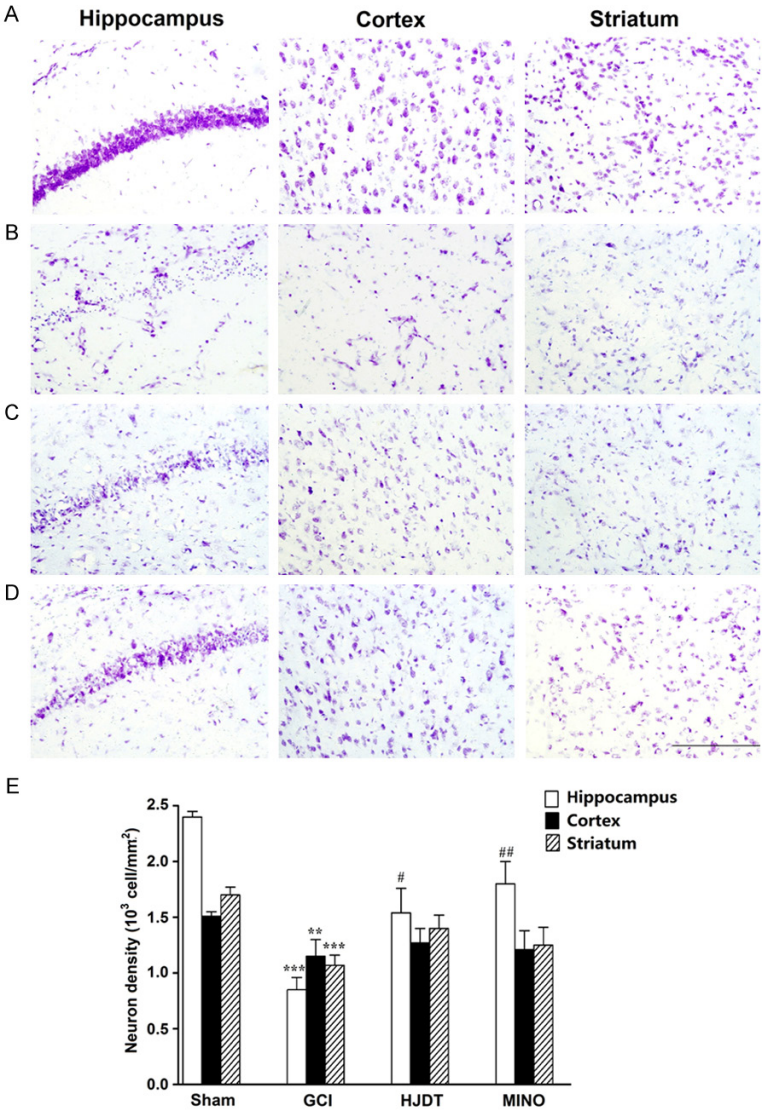

Figure 6. Effects of HJDT on the morphological change of neurons (A-D) and the neuron density (E) in hippocampal CA1 region, cortex and striatum after GCI in mice. (A-D) ×200. Scale bars = 150 μm (n = 8~12, $\bar{x} \pm SEM$). *vs* sham group, $^{**}P < 0.01$, $^{***}P < 0.001$; *vs* GCI group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.

ratio during 15-30 min after administration, but showed no difference in the total traveled distance. This may be related to the extent of recovery after GCI injury, indicating that treatment with only HJDT had no significant improvement in sensorimotor function.

The NOR test has been a widely used method for the investigation into memory alterations [19-21], including GCI [22]. This test is also related with the hippocampal-dependent visual memory [23]. Hartman [22] found that GCI induces both neuronal loss in dorsal hippocampal CA1 region and learning-memory deficits using water maze, object recognition, and radial arm maze. Our study showed the same results in accordance with Hartman reported after GCI. HJDT (4 g/kg) and minocyline significantly increased the DR and DI of the novel object. They also improved the appearance of hippocampal neurons and increased the hippocampal neuron density in the hippocampal CA1 region, but not in the cortex and striatum. These results indicated that HJDT and minocyline could improve the hippocampal-dependent memory deficit after GCI.

Accumulating evidence indicated that inflammation reaction played a key role in the pathogenesis of cerebral ischemia and secondary damage [5, 24, 25]. The inflammatory responses to reperfusion were characterized by a rapid activation of resident cells (mainly microglia), leading to the production of inflammatory cytokines, such as TNF-α and IL-1β [26-29]. The results from this study showed HJDT (4 g/kg) treatment orally for 14 days after GCI reduced the expression of Iba-1, IL-1β, and TNF-α. It indicated the neuroprotective effect of HJDT may be related with inhibition of microglial activation and the induction of IL-1β and TNF-α.

Using HPLC, our previous study found the active components of HJDT are berberine, baicalin, and geniposide [13]. Berberine, baicalin, and geniposide all did show anti-inflammatory effects in different documents. Berberine is known for its anti-inflammatory, anti-oxidative, and anti-microbial effects. Jia L [30] reported berberine treatment significantly inhibited Aβ-stimulated inflammatory cytokine of IL-6 and MCP-1, and also strongly inhibited the NF-κB activation. This indicated berberine was a potent suppressor of neuroflammation. In addition, berberine inhibited the Aβ-induced inflammatory reaction in SH-SY5Y cell lines [31]. Also baicalin effectively down-regulated the expression of macrophage migration inhibitory cytokine in rats [32]. Geniposide inhibited

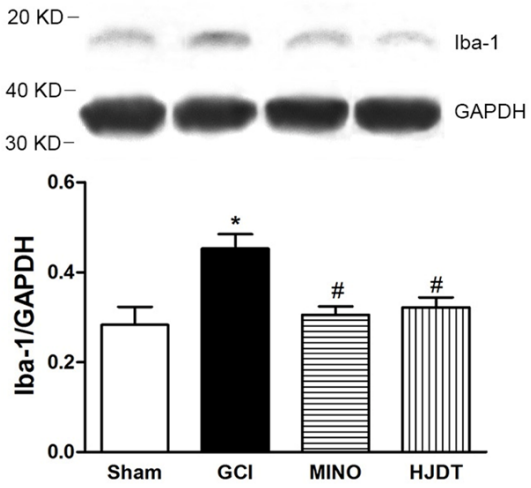

Figure 7. The effect of HJDT on the expression of Iba-1 in the ischemic periphery after GCI injury in mice (n = 3, $\bar{x} \pm SEM$). *vs* sham group, $^{*}P < 0.05$; *vs* GCI group, $^{\#}P < 0.05$.

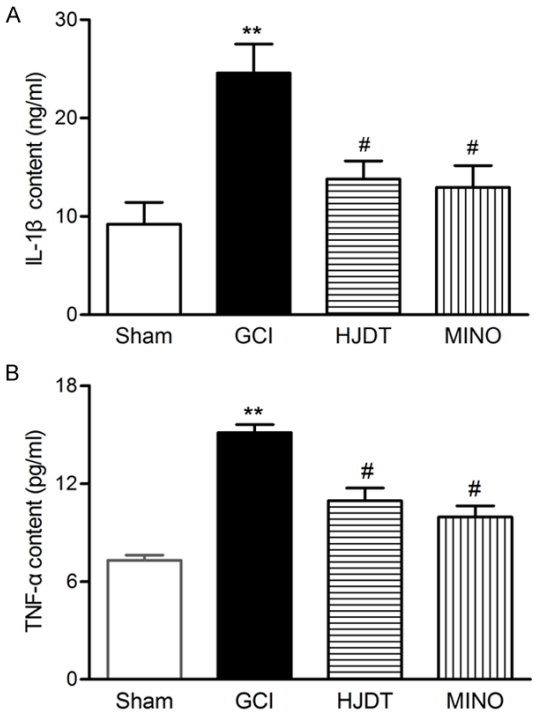

Figure 8. The effect of HJDT on the contents of TNF-α, and IL-1β in the ischemic periphery after GCI injury in mice (n = 3, $\bar{x} \pm SEM$). *vs* sham group, $^{**}P < 0.01$; *vs* GCI group, $^{\#}P < 0.05$.

Aβ-induced microglial activation and lead to the reduction of proinflammatory cytokines, such as TNF-α and IL-1β in Alzheimer's disease [33]. These results suggested that berberine, baicalin, and geniposide may be responsible for the anti-inflammatory effect of HJDT on GCI.

Acknowledgements

This work was supported by grants from the Access Engineer Project of Zhejiang Province (FG2016010) and the Foundation of Science Technology Department of Zhejiang Province (2016C37099).

Disclosure of conflict of interest

None.

Address correspondence to: Qi Zhang, Department of Pharmacology, Zhejiang Medical College, Hangzhou 310053, China. Tel: +86 571 87692627; Fax: +86 571 87692627; E-mail: zhangqi@hmc.edu.cn

References

[1] Alexandrov AV. Current and future recanalization strategies for acute ischemic stroke. J Intern Med 2010; 267: 209-219.

[2] Amantea D, Nappi G, Bernardi G, Bagetta G and Corasaniti MT. Post-ischemic brain damage: pathophysiology and role of inflammatory mediators. FEBS J 2009; 276: 13-26.

[3] McColl BW, Allan SM and Rothwell NJ. Systemic inflammation and stroke: aetiology, pathology and targets for therapy. Biochem Soc Trans 2007; 35: 1163-1165.

[4] Eggen BJ, Raj D, Hanisch UK and Boddeke HW. Microglial phenotype and adaptation. J Neuroimmune Pharmacol 2013; 8: 807-823.

[5] Briones TL, Woods J and Wadowska M. Chronic neuroinflammation and cognitive impairment following transient global cerebral ischemia: role of fractalkine/CX3CR1 signaling. J Neuroinflammation 2014; 11: 13.

[6] Nakajima K and Kohsaka S. Microglia: neuroprotective and neurotrophic cells in the central nervous system. Curr Drug Targets Cardiovasc Haematol Disord 2004; 4: 65-84.

[7] Ohta Y, Kobayashi T, Nishida K, Sasaki E and Ishiguro I. Preventive effect of Oren-gedoku-to (Huanglian-Jie-Du-Tang) extract on the development of stress-induced acute gastric mucosal lesions in rats. J Ethnopharmacol 1999; 67: 377-384.

[8] Ohta Y, Kongo-Nishimura M, Hayashi T and Kishikawa T. Effect of Oren-gedoku-to (Huanglian-Jie-Du-Tang) extract on disruption of hepatic antioxidant defense systems in rats treated with D-galactosamine. J Ethnopharmacol 2004; 94: 323-329.

[9] Durairajan SS, Huang YY, Yuen PY, Chen LL, Kwok KY, Liu LF, Song JX, Han QB, Xue L, Chung SK, Huang JD, Baum L, Senapati S and Li M. Effects of Huanglian-Jie-Du-Tang and its modi-

fied formula on the modulation of amyloid-beta precursor protein processing in Alzheimer's disease models. PLoS One 2014; 9: e92954.
[10] Xu J, Murakami Y, Matsumoto K, Tohda M, Watanabe H, Zhang S, Yu Q and Shen J. Protective effect of Oren-gedoku-to (Huang-Lian-Jie-Du-Tang) against impairment of learning and memory induced by transient cerebral ischemia in mice. J Ethnopharmacol 2000; 73: 405-413.
[11] Hwang YS, Shin CY, Huh Y and Ryu JH. Hwangryun-Hae-Dok-tang (Huanglian-Jie-Du-Tang) extract and its constituents reduce ischemia-reperfusion brain injury and neutrophil infiltration in rats. Life Sci 2002; 71: 2105-2117.
[12] Zeng MF, Pan LM, Zhu HX, Zhang QC and Guo LW. Comparative pharmacokinetics of baicalin in plasma after oral administration of Huang-Lian-Jie-Du-Tang or pure baicalin in MCAO and sham-operated rats. Fitoterapia 2010; 81: 490-496.
[13] Ye Y, Huang C, Jiang L, Shen X, Zhu S, Rao Y, Wang J and Zhang Q. Huanglian-Jie-Du-Tang extract protects against chronic brain injury after focal cerebral ischemia via hypoxia-inducible-factor-1alpha-regulated vascular endothelial growth factor signaling in mice. Biol Pharm Bull 2012; 35: 355-361.
[14] Bernard SA, Gray TW, Buist MD, Jones BM, Silvester W, Gutteridge G and Smith K. Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. N Engl J Med 2002; 346: 557-563.
[15] Salazar JD, Wityk RJ, Grega MA, Borowicz LM, Doty JR, Petrofski JA and Baumgartner WA. Stroke after cardiac surgery: short- and long-term outcomes. Ann Thorac Surg 2001; 72: 1195-1201; discussion 1201-1192.
[16] Murakami K, Kondo T, Kawase M and Chan PH. The development of a new mouse model of global ischemia: focus on the relationships between ischemia duration, anesthesia, cerebral vasculature, and neuronal injury following global ischemia in mice. Brain Res 1998; 780: 304-310.
[17] Zhang Q, Wei EQ, Zhu CY, Zhang WP, Wang ML, Zhang SH, Yu YP and Chen Z. Focal cerebral ischemia alters the spatio-temporal properties, but not the amount of activity in mice. Behav Brain Res 2006; 169: 66-74.
[18] Milot MR and Plamondon H. Time-dependent effects of global cerebral ischemia on anxiety, locomotion, and habituation in rats. Behav Brain Res 2009; 200: 173-180.
[19] Moore SJ, Deshpande K, Stinnett GS, Seasholtz AF and Murphy GG. Conversion of short-term to long-term memory in the novel object recognition paradigm. Neurobiol Learn Mem 2013; 105: 174-185.
[20] Paris JJ, Eans SO, Mizrachi E, Reilley KJ, Ganno ML and McLaughlin JP. Central administration of angiotensin IV rapidly enhances novel object recognition among mice. Neuropharmacology 2013; 70: 247-253.
[21] Hansen KF, Sakamoto K, Wayman GA, Impey S and Obrietan K. Transgenic miR132 alters neuronal spine density and impairs novel object recognition memory. PLoS One 2010; 5: e15497.
[22] Hartman RE, Lee JM, Zipfel GJ and Wozniak DF. Characterizing learning deficits and hippocampal neuron loss following transient global cerebral ischemia in rats. Brain Res 2005; 1043: 48-56.
[23] Stanley EM, Wilson MA and Fadel JR. Hippocampal neurotransmitter efflux during one-trial novel object recognition in rats. Neurosci Lett 2012; 511: 38-42.
[24] Okuyama S, Morita M, Miyoshi K, Nishigawa Y, Kaji M, Sawamoto A, Terugo T, Toyoda N, Makihata N, Amakura Y, Yoshimura M, Nakajima M and Furukawa Y. 3,5,6,7,8,3',4'-Heptamethoxyflavone, a citrus flavonoid, on protection against memory impairment and neuronal cell death in a global cerebral ischemia mouse model. Neurochem Int 2014; 70: 30-38.
[25] Kuang G, He Q, Zhang Y, Zhuang R, Xiang A, Jiang Q, Luo Y and Yang J. Modulation of preactivation of PPAR-beta on memory and learning dysfunction and inflammatory response in the hippocampus in rats exposed to global cerebral ischemia/reperfusion. PPAR Res 2012; 2012: 209794.
[26] Yeh CH, Shih HC, Hong HM, Lee SS, Yang ML, Chen CJ and Kuan YH. Protective effect of wogonin on proinflammatory cytokine generation via Jak1/3-STAT1/3 pathway in lipopolysaccharide stimulated BV2 microglial cells. Toxicol Ind Health 2015; 31: 960-6.
[27] Zhang P, Liu X, Zhu Y, Chen S, Zhou D and Wang Y. Honokiol inhibits the inflammatory reaction during cerebral ischemia reperfusion by suppressing NF-kappaB activation and cytokine production of glial cells. Neurosci Lett 2013; 534: 123-127.
[28] Abulafia DP, de Rivero Vaccari JP, Lozano JD, Lotocki G, Keane RW and Dietrich WD. Inhibition of the inflammasome complex reduces the inflammatory response after thromboembolic stroke in mice. J Cereb Blood Flow Metab 2009; 29: 534-544.
[29] Barone FC, Arvin B, White RF, Miller A, Webb CL, Willette RN, Lysko PG and Feuerstein GZ. Tumor necrosis factor-alpha. A mediator of focal ischemic brain injury. Stroke 1997; 28: 1233-1244.

[30] Jia L, Liu J, Song Z, Pan X, Chen L, Cui X and Wang M. Berberine suppresses amyloid-beta-induced inflammatory response in microglia by inhibiting nuclear factor-kappaB and mitogen-activated protein kinase signalling pathways. J Pharm Pharmacol 2012; 64: 1510-1521.

[31] Xu J, Zhang H, Yang F and Yu JX. [Intervention effect of berberine on expressions of TNF-alpha and receptor type I in Abeta25-35-induced inflammatory reaction in SH-SY5Y cell lines]. Zhongguo Zhong Yao Za Zhi 2013; 38: 1327-1330.

[32] Dai SX, Zou Y, Feng YL, Liu HB and Zheng XB. Baicalin down-regulates the expression of macrophage migration inhibitory factor (MIF) effectively for rats with ulcerative colitis. Phytother Res 2012; 26: 498-504.

[33] Lv C, Wang L, Liu X, Cong X, Yan SS, Wang Y and Zhang W. Geniposide attenuates oligomeric Abeta(1-42)-induced inflammatory response by targeting RAGE-dependent signaling in BV2 cells. Curr Alzheimer Res 2014; 11: 430-440.