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Original Article

Icariin administration is associated with enhanced homing of bone marrow-derived progenitor cells, increased levels of CXCR-4/SDF-1α homing factors, and attenuated brain injury in a rat stroke model

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Abstract: The goal of this study was to investigate the mechanisms of neuroprotection by Icariin (ICA) treatment against brain ischemia in a rat stroke model via enhanced homing of bone marrow-derived progenitor cells and modulation of the CXCR-4/SDF-1α homing factor axis. Infarct size, brain water content, and neurological deficits by neurobehavioral scoring were evaluated in a middle cerebral artery occlusion model in rats treated with ICA or saline as a control. mRNA expression and protein levels of SDF-1α and CXCR-4 were investigated by quantitative reverse transcription polymerase chain reaction and Western blot analysis. Fluorescence-activated cell sorting analysis was performed to determine the effects of ICA on bone marrow cells. Neurological scores, infarct size, and brain edema all significantly improved following ICA treatment ($P < 0.05$). In comparison with the saline control group, $CD45^+/CD34^+$, $CD45^+/CD34^+/CD31^+$, and $CD45^+/CD34^+/c\text{-}kit^+$ bone marrow-derived stem cells were increased in the peripheral blood ($P < 0.05$), while $CD45^+/CD34^+$ and $CD45^+/CD34^+/c\text{-}kit^+$ stem cells were elevated in bone marrow in the ICA treatment group ($P < 0.05$). $CD45^+/CD34^+/CD31^+$ cells showed only a slight, but not significant, increase in bone marrow in ICA-treated rats. Compared with the control group, protein, and mRNA levels of the homing factors, SDF-1α and CXCR-4, were higher with ICA treatment ($P < 0.05$), peaking after seven days and remaining elevated for at least 14 days following stroke. ICA protects against brain ischemic injury likely by enhancing the number of mobilized bone marrow-derived progenitor cells, which may play a key role in the repair of ischemic brain damage, and by elevating CXCR-4/SDF-1α homing factor levels.

Keywords: Icariin, bone marrow-derived stem cells, CXCR-4/SDF-1α homing factors, neuroprotection, ischemic stroke

Introduction

Ischemic stroke has become a leading cause of long-term disability and mortality [1]. Currently, pharmacological treatment is not available to prevent neuron death and degeneration triggered by ischemia and reperfusion. Administration of bone marrow-derived mesenchymal stem cells (BMSCs) can improve the functional outcome for stroke in rats [2]. Studies using granulocyte-colony stimulating factor (G-CSF) in combination with BMSCs revealed an improvement in neurological function and survival in a stroke model in rats. This was accompanied by robust angiogenesis in the infarct core and the surrounding region [3]. However, G-CSF in combination with BMSC treatment failed to impact spatial reference-memory or infarct volume in this study.

Stromal cell-derived factor-1α (SDF-1α) and its cellular receptor CXCR4 have been demonstrated to be pivotal elements for stem-cell mobilization and homing in many species and tissue types [4, 5]. SDF-1α/CXCR4 plays a key role in the development of the cerebellum [6], the cerebral cortex [7], the dentate gyrus [8], and motor axons [9]. In some hypoxic conditions, such as ischemic cardiomyopathy [10] and renal ischemia/reperfusion injury [11], SDF-1α

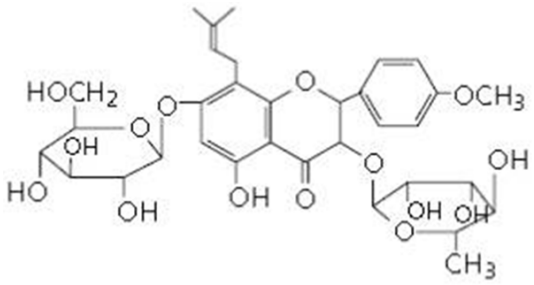

Figure 1. Structure of icariin (2-(4'-methoxylphenyl)-3-rhamnosido-5-hydroxyl-7-glucosido-8-(3'-methyl-2-butylenyl)-4-chromanone).

and CXCR4 are significantly elevated. These results raise the possibility that the SDF-1α/CXCR4 axis might play a crucial role in regulating BMSC migration in the ischemic brain.

Icariin (ICA) (2-(4'-methoxylphenyl)-3-rhamnosido-5-hydroxyl-7-glucosido-8-(3'methyl-2-butylenyl)-4-chromanone) (**Figure 1**), a flavonol isolated from Epimedii herba, is considered the primary active component of Epimedium extracts. Epimedium is a traditional Chinese herb, which has been extensively used for more than one thousand years in China. Because of its effect on increasing cerebral blood flow, ICA has been widely used for the treatment of stroke in Chinese traditional medicine [12]. ICA has the ability to improve spatial learning and memory abilities in rats with brain dysfunction due to decreased expression of TNF-alpha, IL-1 and COX-2 in the hippocampus [13]. However, it has not been investigated whether the neuroprotective ability of ICA is associated with enhanced mobilization and homing of BMSCs via the CXCR-4/SDF-1α axis.

Therefore, in this study, rats were treated with ICA before performing middle cerebral artery occlusion (MCAO) in rats, and effects on infarct size, brain water content and neurological deficits by neurobehavioral scoring, as well as on the number of mobilized BMSCs and CXCR-4/SDF-1 expression were evaluated.

Materials and methods

MCAO model for stroke in rats

Adult male Sprague-Dawley rats (250-300 g) were purchased from the Shanghai Laboratory Animal Center, Chinese Academy of Sciences. The animals were housed in groups of eight and maintained in controlled temperature (22 ± 2°C) and humidity (60-70%), and under a 12 hour light-dark cycle (lights on 07:00 AM). Food and water were available ad libitum. The MCAO model for stroke was established as described [14]. Briefly, rats were anesthetized with pentobarbital (1%, 50 mg/kg), and the middle cerebral artery was occluded permanently using a piece of 6-0 monofilament nylon suture. Successful occlusion was confirmed by an 87-90% reduction in cerebral blood flow, as measured by Laser-Doppler flowmetry. Mortality was around 10%. In all experimental protocols, the animals were monitored twice daily for signals of severe distress. The criteria adopted to decide on euthanasia included the occurrence of convulsions, and/or severe abdominal distension. All procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use the National Research Council's Guide for the Humane Care and Use of Laboratory Animals. The experimental protocol was approved by the Ethics Committee of Jiangsu Province Hospital of Traditional Chinese Medicine, China.

Drug treatment with ICA

In accordance with previous reports [13, 15], three doses of ICA (30, 60 and 120 mg/kg) were chosen for preliminary experiments. The results showed that based on the infarction size of the brain tissue, 120 mg/kg ICA was the optimal dose for protection against brain ischemia injury. In the following study, 81 rats were equally and randomly divided into three groups: sham group, MCAO with ICA group, and MCAO with vehicle group. Rats in the ICA group were treated with ICA (purity > 98%; Sulang Pharmaceutic Technology Co., LTD, Nanjing, China) through intragastric administration at a dose of 120 mg/kg per day for 2 weeks before MCAO. Physiological saline solution was used as the vehicle treatment. For further analysis, nine rats of each group were deeply anesthetized with pentobarbital (1%, 50 mg/kg) and euthanized by decapitation days 3, 7, or 14 post injury.

Infarct size measurement in MCAO rats

Nine rats of each group were selected for infarct size measurement. The infarct size after MCAO was determined by 2, 3, 5-Triphenyl-2H-tetrazolium chloride (TTC) staining (Sigma, USA)

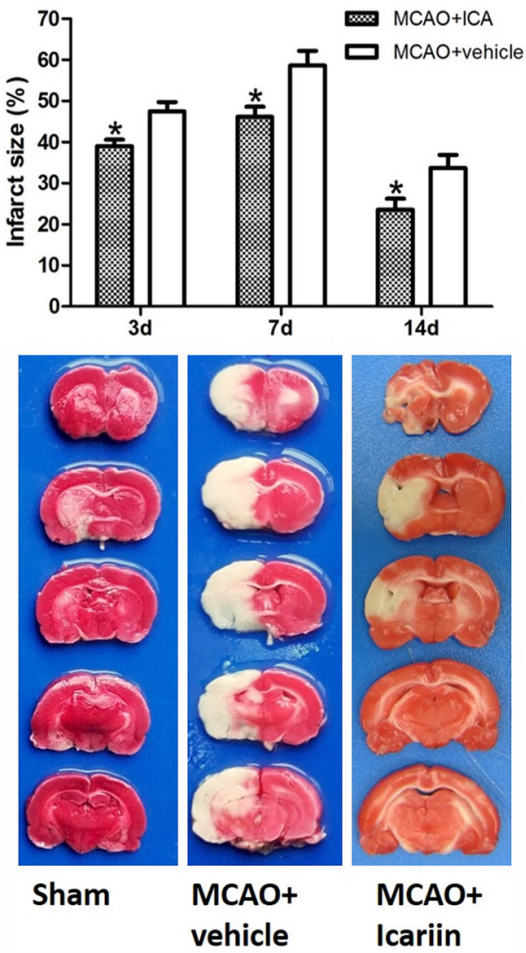

Figure 2. Effect of ICA treatment on the infarction size in MCAO rats. Three rats of each group were used for infarction size measurement at each time point. The infarction size in the ICA treatment group was significantly decreased compared with the vehicle-treated group at all time points measured (day 3: 38.98% ± 1.62 vs. 47.43% ± 2.32; day 7: 46.12% ± 2.46 vs. 58.62% ± 3.52; day 14: 23.55% ± 2.65 vs. 33.67% ± 3.18; $P < 0.05$). *$P < 0.05$ vs. vehicle group.

at days 3, 7 and 14 as described previously [16]. TTC stained the normal brain tissue deep red, while the infarct area was stained a pale gray color. Infarction area and hemispheric areas of each slice were photographed and analyzed using an image-analysis software Osiris (version 4.19, University Hospital of Geneva, Switzerland). The infarct volume in all slices was expressed as a percentage of the contralateral hemisphere after correcting for edema [17].

Nissl staining

Nissl staining was performed 14 days after MCAO. Sections (4 mm) were hydrated in 1% toluidine blue at 50°C for 20 min. After rinsing with double distilled water, they were dehydrated and mounted with Permount. Images of the cortex and CA1 area of the hippocampus from each animal were captured and Imaging-Pro-Plus software (Leika DMLB, Leica, Wetzlar, Germany) was used to perform quantitative analysis of cell numbers.

Neurobehavioral evaluation of MCAO rats

To evaluate the neurological damage in MCAO rats, the performance of each rat was rated according to neurological deficit scores, which were described previously [18].

Brain water content in MCAO rats

Animals were anesthetized and sacrificed by decapitation at different time points (days 3, 7 and 14). The brains were quickly removed, weighed to obtain the wet weight, dried at 100°C for 24 h, and then weighed on an analytical balance (Sartorius AG, Goettingen, Germany) to obtain the brain water content. The formula for calculating the water content was the following: water content (%) = 100 × (wet weight-dry weight)/wet weight.

Western blot analysis

Proteins were extracted from the cortex according to procedures described previously [11]. Membranes were probed with primary antibodies against SDF-1α and CXCR4 (1:1000, Santa Cruz Biotech, Santa Cruz, CA, USA) followed by horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibodies. Immunostained protein bands were visualized using the enhanced chemiluminescence method with an ECL kit (Amersham Pharmacia Biotech, Piscataway, NJ). The intensity of protein bands was quantified by densitometry using Image J software (NIH, Bethesda, MA). The loading control was β-actin, and relative expression of proteins was normalized to β-actin levels.

Real-time quantitative RT-PCR

Total cellular RNAs from rats' brain cortex were extracted using TRIzol reagent (GIBCO-BRL, Waltham, MA, USA) and reversed into cDNA using AMV reverse transcriptase (Invitrogen, Carlsbad, CA, USA). Real-time quantitative RT-PCR (qRT-PCR) was performed with the ABI Prism 7300 sequence detection system (App-

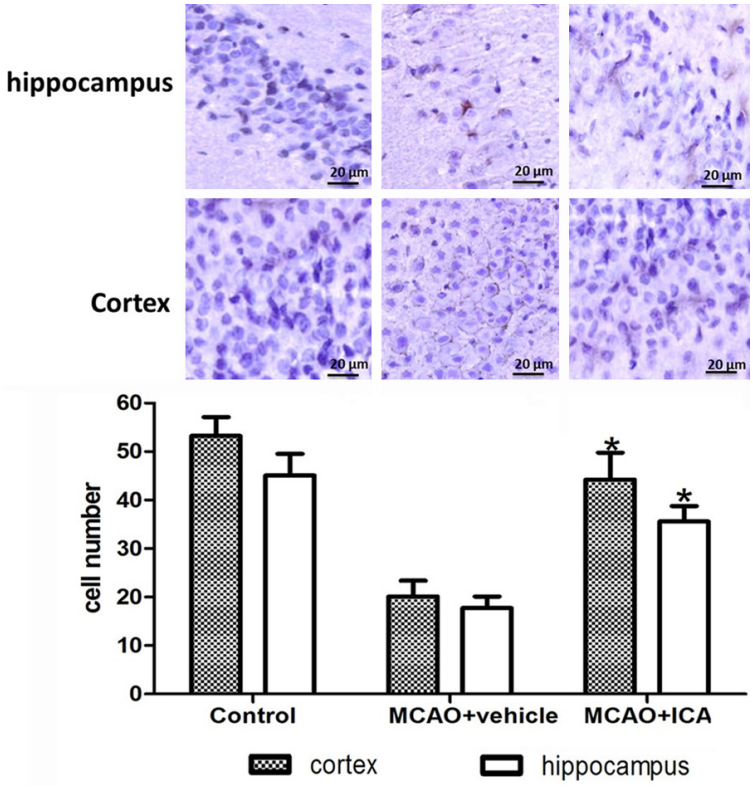

Figure 3. Effect of ICA treatment on brain cell damage in MCAO rats. In the sham and ICA group, the cell outline was clear and cell structure was compact. Cells were large and had abundant cytoplasm and Nissl body staining. In the vehicle group, cells were arranged sparsely and the cell outline was fuzzy. The number of cells with eumorphism in the ICA group was significantly reduced. More Nissl-stained cells were observed in the ICA group compared with the vehicle-treated group on day 14 after MCAO (cortex: 44.2 ± 5.6 vs. 20.1 ± 3.3; hippocampus: 35.6 ± 3.2 vs. 17.7 ± 2.4; $P < 0.05$). *$P < 0.05$ vs. vehicle group.

lied Biosystems, Foster City, CA, USA) using Taqman probes (Shanghai Shinegene Molecular Biotechnology Co. Ltd, Shanghai, China). β-actin was used as housekeeping gene for internal normalization. The transcript copy number of the target genes was determined on the basis of their Ct values. The fold change in mRNA expression was obtained using the $2_T^{\Delta\Delta Ct}$ method.

Flow cytometry

Flow cytometry was used to measure the levels of BMSCs in peripheral blood, bone marrow, and brain tissue. At days 3, 7, and 14 after MCAO, 1 ml of peripheral blood was harvested from the caudal vein of the animals in each group. Bone marrow cells were obtained by flushing the tibiae and femurs from euthanized rats. Mononuclear cells were separated by density-gradient centrifugation using 1.077 g/ml Histopaque solution (Sigma-Aldrich, St. Louis, MO, USA), purified, and re-suspended in phosphate buffered saline containing 1% bovine serum albumin. Cells were incubated for 40 minutes in the dark at 4°C withfluoresceinisothiocyanate(FITC), phycoerythrin (PE), or peridinin chlorophyll-protein (PerCP) conjugated monoclonal antibodies CD45-PerCP, CD34-FITC, CD31-PE and c-kit-PE (all from BD Biosciences Pharmingen, San Diego, CA, USA). Matching isotype antibodies (BD Biosciences Pharmingen) served as controls. Cells were analyzed by three-color fluorescence-activated cell sorting (FACS) using a Coulter Epics XLMCLTM flow cytometer (Beckman Coulter, Fullerton, CA, USA). Each analysis included 50000 events.

Statistical analysis

The data are expressed as mean ± standard deviation (SD) and were analyzed using the SPSS 20.0 statistical analysis software (SPSS, Chicago, IL, USA). Differences between multiple groups were noted by one-way analysis of variance (ANOVA). Comparisons between two groups were performed using the Student's t test. $P < 0.05$ was considered as statistically significant.

Results

Protective effect of ICA treatment in the MCAO model of stroke in rats

The visual evaluation of the infarction size (grey areas) in the TTC-stained coronal sections revealed a noticeably reduced size in ICA-treated rats compared with sham-operated and vehicle-treated rats (**Figure 2**). The results of image quantification showed that the infarction size in the ICA treatment group was significantly decreased compared with the vehicle-treated group at all of the time points measured (**Figure 2**, day 3: 38.98% ± 1.62 vs. 47.43% ± 2.32; day 7: 46.12% ± 2.46 vs. 58.62% ± 3.52; day 14:

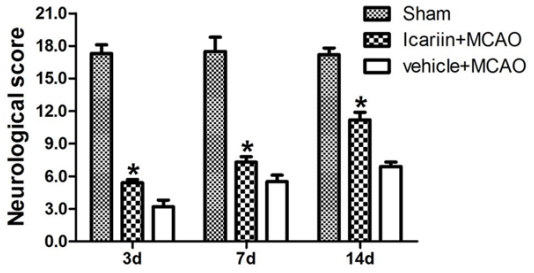

Figure 4. Effect of ICA treatment on neurological deficits in MCAO rats. Ischemia-induced neurological deficits were significantly ameliorated in rats that received ICA treatment compared with vehicle-treated rats at days 3, 7, and 14 after MCAO (day 3: 5.4 ± 0.3 vs. 3.2 ± 0.6; day 7: 7.3 ± 0.5 vs. 5.5 ± 0.6; day 14: 11.2 ± 0.7 vs. 6.9 ± 0.4; $P < 0.05$ for all conditions). *$P < 0.05$ vs. vehicle group.

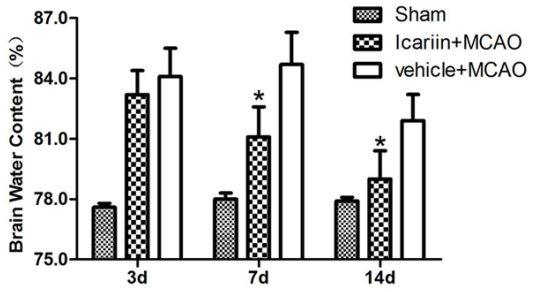

Figure 5. Effect of ICA treatment on brain water content in MCAO rats. Quantification of the cerebral water content was less in the ICA group compared with the vehicle group (day 3: 83.2% ± 1.2 *vs.* 84.1% ± 1.4; day 7: 81.1% ± 1.5 *vs.* 84.7% ± 1.6; day 14: 79.0% ± 1.4 *vs.* 81.9% ± 1.3; $P < 0.05$ at day 7 and 14). *$P < 0.05$ *vs.* vehicle group.

23.55% ± 2.65 vs. 33.67% ± 3.18; $P < 0.05$). Extensive neuronal changes were noticed in the cortex and CA1 region of the hippocampus by Nissl staining with features, such as considerable dark, pyknotic neurons, in the vehicle-treated group. More Nissl-stained cells were observed in the ICA group compared with the vehicle-treated group on day 14 after MCAO (**Figure 3**, cortex: 44.2 ± 5.6 vs. 20.1 ± 3.3; hippocampus: 35.6 ± 3.2 vs. 17.7 ± 2.4; $P < 0.05$).

The scores ranged from the lowest score of three to a maximum score of 18. In vehicle-treated MCAO rats, the neurological score decreased to a score of 3.2 after three days, and gradually improved to a score of 6.9 after 14 days. The neurological scores for MCAO rats treated with ICA significantly improved compared to vehicle-treated rats (**Figure 4**, day 3:

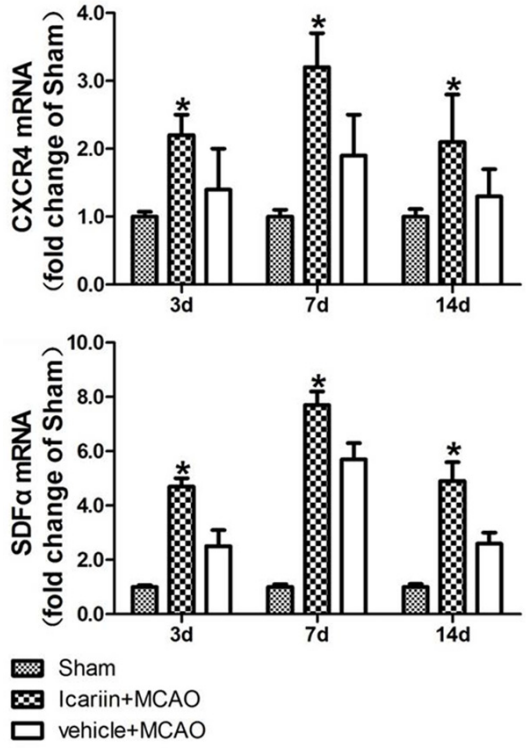

Figure 6. Effect of ICA treatment on mRNA expression of SDF-1 and CXCR4 evaluated by qRT-PCR in MCAO rats. ICA administration significantly increased the mRNA expression of SDF-1α and CXCR4 in MCAO rats at all time points (1.8, 1.4, and 1.9-fold relative to sham control versus 1.6, 1.7, and 1.6-fold in the MCAO + vehicle group at days 3, 7, and 14, respectively, $P < 0.05$ compared with MCAO + vehicle). *$P < 0.05$ vs. MCAO + vehicle group (n = 9).

5.4 ± 0.3 vs. 3.2 ± 0.6; day 7: 7.3 ± 0.5 vs. 5.5 ± 0.6; day 14: 11.2 ± 0.7 vs. 6.9 ± 0.4; $P < 0.05$ for all conditions). The cerebral water content, which is another measure of stroke-associated brain damage, was increased in vehicle-treated MCAO rats compared with the sham-operated controls (**Figure 5**). ICA-treatment led to a significant reduction of the brain water content compared with the vehicle group at day 7 and 14 after stroke induction (**Figure 5**, day 3: 83.2% ± 1.2 vs. 84.1% ± 1.4; day 7: 81.1% ± 1.5 vs. 84.7% ± 1.6; day 14: 79.0% ± 1.4 vs. 81.9% ± 1.3; $P < 0.05$ at day 7 and 14).

ICA-treatment up-regulates SDF-1α and CXCR4 protein and mRNA levels

The vehicle-treated group had a marked increase in SDF-1α and CXCR4 mRNA levels at days 3, 7 and 14 (**Figure 6**). However, ICA administration significantly increased their expression

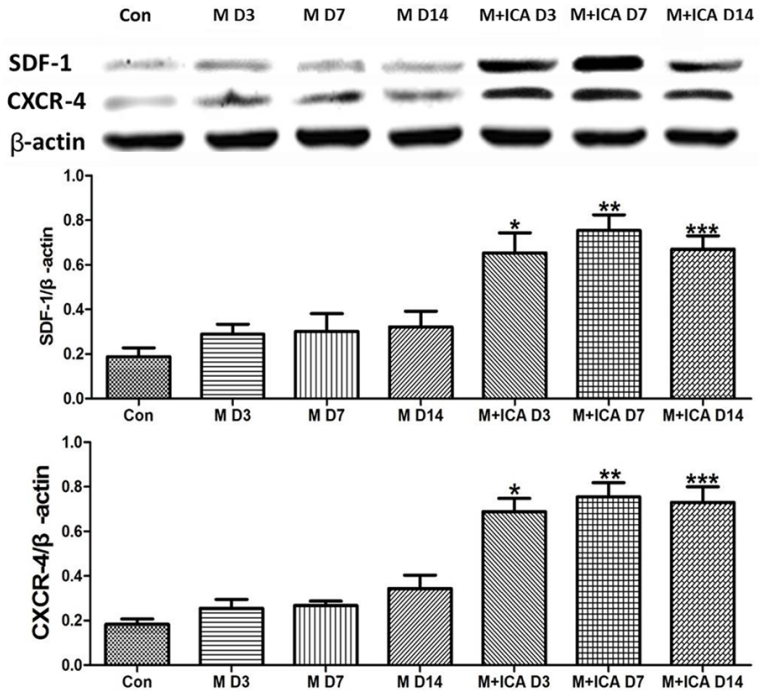

Figure 7. Effect of ICA treatment on SDF-1 and CXCR4 protein levels in MCAO rats. Quantification of the β-actin-normalized densities of the immunoreactive bands revealed significantly increased expression of SDF-1α and CXCR4 in MCAO rats at all of the time points measured (D3, D7, and D14) compared to the vehicle group. Densitometric quantification using β-actin normalized values showed a 1.96, 1.86, and 1.69-fold increase at days 3, 7, and 14, respectively, in the ICA group compared to the vehicle treatment group. CXCR4 protein levels were 3.28, 2.15, and 2.38-fold increased after ICA treatment at the same time points as compared with vehicle-treated controls ($P < 0.05$). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{*}P < 0.001$ vs. vehicle group ($n = 9$).

over that of the vehicle-treated group (1.8, 1.4, and 1.9-fold relative to sham control versus 1.6, 1.7, and 1.6-fold in the MCAO + vehicle group at days 3, 7, and 14, respectively, $P < 0.05$ compared with MCAO + vehicle).

Western blot analysis revealed that the protein levels of SDF-1α and CXCR4 were both increased in the vehicle-treated control group compared to the sham-operated group, and ICA treatment resulted in further elevated protein levels (**Figure 7**). Densitometric quantification using β-actin normalized values showed a 1.96, 1.86, and 1.69-fold increase at days 3, 7, and 14, respectively, in the ICA group compared to the vehicle treatment group. The CXCR4 protein levels were 3.28, 2.15, and 2.38-fold increased after ICA treatment at the same time points as compared with vehicle-treated controls ($P < 0.05$).

ICA-treatment increases the number of BMSCs in peripheral blood, bone marrow and brain tissue of MCAO rats

To investigate the effect of ICA on the mobilization of BMSCs in peripheral blood, bone marrow and brain tissue, we performed flow cytometry using different immune markers for subpopulations of mononuclear cells (**Figure 8**). We found a significant increase in the numbers of different subtypes of $CD45^+/CD34^+$ cells in the peripheral blood after ICA administration. $CD45^+/CD34^+$ cells displayed a 3.7, 4.2, and 4.1-fold increase, $CD45^+/CD34^+/CD31^+$ cells had a 3.3, 5.1, and 5.5-fold increase, and $CD45^+/CD34^+$/c-kit cells had a 1.4, 1.7, and 2.2-fold increase at day 3, 7, and 14, respectively ($P < 0.05$ for all conditions) (**Figure 8**). A representative FACS analysis visualized the difference in the number of $CD45^+/CD34^+$ cells in peripheral blood of ICA or vehicle-treated MCAO rats (**Figure 8B**).

In bone marrow, the number of $CD45^+/CD34^+$ cells significantly increased 1.4, 1.3, and 1.5-fold, and $CD45^+/CD34^+/c\text{-}Kit^+$ cells increased numbers by 1.5, 1.4, and 1.2-fold at days 3, 7, and 14, respectively ($P < 0.05$ for all conditions) (**Figure 8**).

In brain tissue, $CD45^+/CD34^+$ cells displayed a 6.5, 4.8, and 4.1-fold increase, $CD45^+/CD34^+/CD31^+$ cells had a 4.8, 4.9, and 5.8-fold increase, and $CD45^+/CD34^+$/c-kit cells had a 2.4, 3.3, and 3.5-fold increase at days 3, 7, and 14 after ICA treatment, respectively ($P < 0.05$ for all conditions) (**Figure 8**).

Discussion

In the present study, the protective effects of ICA in the preclinical MCAO rat model for stroke were examined. We particularly focused on homing mechanisms for mobilized BMSCs. The

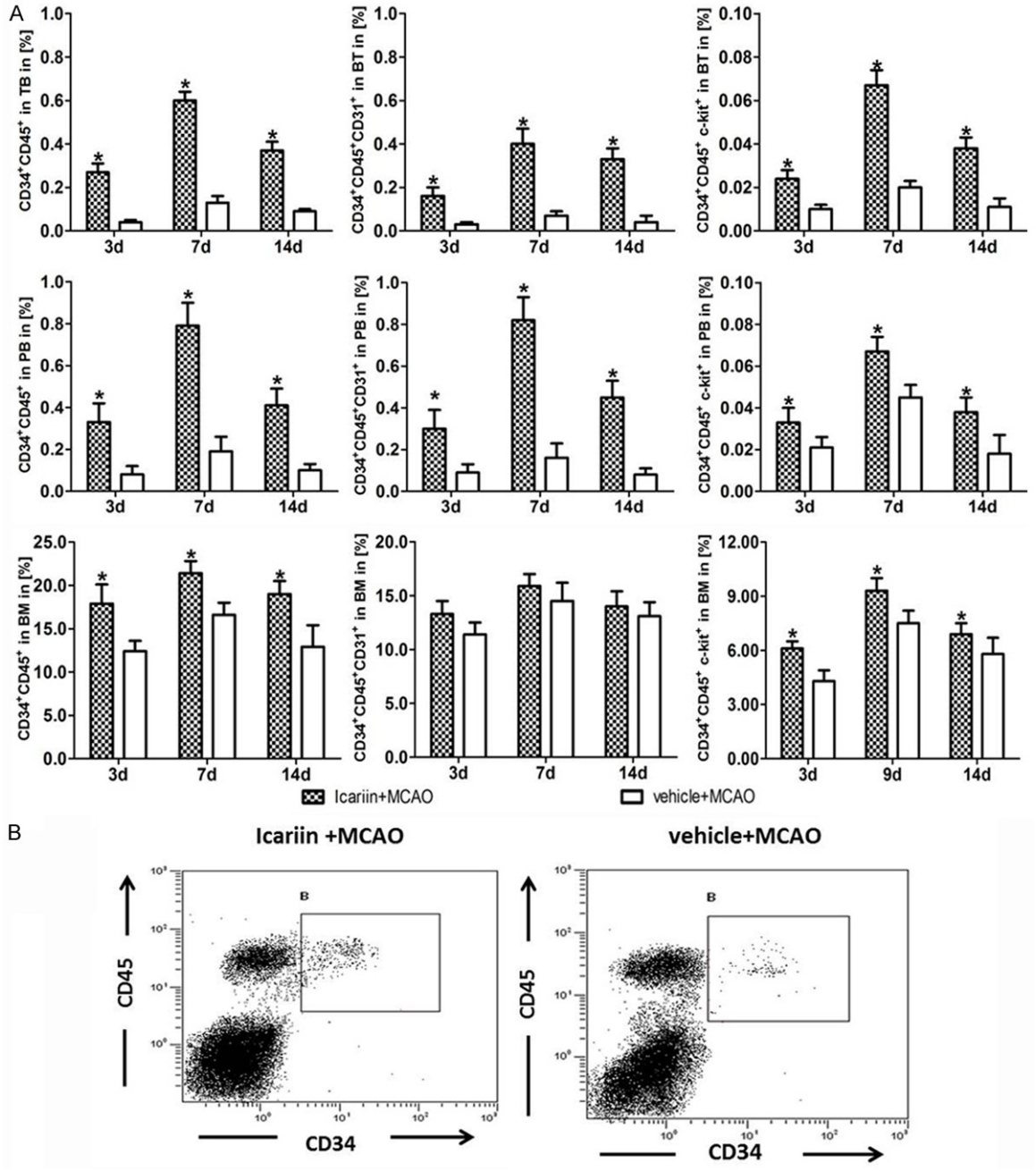

Figure 8. Effect of ICA treatment on the number of $CD45^+/CD34^+$, $CD45^+/CD34^+/CD31^+$ and $CD45^+/CD34^+/c\text{-}Kit^+$ cells at days 3, 7, and 14 in MCAO rats. A. A significant increase of different subtypes of $CD45^+/CD34^+$ (3.7, 4.2, and 4.1-fold increase), $CD45^+/CD34^+/CD31^+$ (3.3, 5.1, and 5.5-fold increase), and $CD45^+/CD34^+/c\text{-}Kit^+$ (1.4, 1.7, and 2.2-fold increase) cells in peripheral blood (PB) at days 3, 7, and 14 after MCAO. In bone marrow (BM), $CD45^+/CD34^+$ (1.4, 1.3, and 1.5-fold increase), and $CD45^+/CD34^+/c\text{-}Kit^+$ (1.5, 1.4, and 1.2-fold increase) cells were significantly increased after ICA administration. In brain tissue (BT), $CD45^+/CD34^+$ cells displayed a 6.5, 4.8, and 4.1-fold increase, $CD45^+/CD34^+/CD31^+$ cells had a 4.8, 4.9, and 5.8-fold increase, and $CD45^+/CD34^+/c\text{-}Kit$ cells had a 2.4, 3.3, and 3.5-fold increase at days 3, 7, and 14 after ICA treatment. $^*P < 0.05$ vs. vehicle group (n = 9). B. Representative FACS analysis of $CD45^+/CD34^+$ cells in peripheral blood of rat.

main findings indicate 1) ICA was protective against ischemic brain injury as revealed by the size of infarcted tissue, scoring neurological deficits and measuring the cerebral water content; 2) ICA increased the expression of the homing factors CXCR-4 and SDF-1; 3) mobiliza-

tion of subpopulations of BMSCs into the peripheral blood occurred without depleting the bone marrow.

The traditional Chinese herb Epimedium has been reported to possess various important pharmacological effects, such as increasing cerebral blood flow, anti-inflammatory effects, promoting nerve regeneration, improving blood circulation, regulating immunity, preventing osteoporosis, and anti-aging effects [19, 22]. Recently, Zhu et al. [23] reported that ICA could protect against brain ischemic injury by increasing expression of SIRT1 and PGC-1a. For the present study, a dose of 120 mg/kg ICA per day for two weeks before MCAO was chosen based on preliminary experiments. The brain infarction area, brain water content, neurological function scores, and the expression levels of the homing factors SDF-1α and CXCR-4 and the mobilization of BMSCs into peripheral blood were analyzed to evaluate the effect of ICA at days 3, 7, 14 after stroke induction. Our results indicate that ICA ameliorates brain injury in MCAO, which resulted in a smaller area of brain infarction, less brain water content, and improved neurological function scores compared to the vehicle group at the three time points examined.

Mobilization of different subpopulations of BMCs was detected in the peripheral blood in the rats of the ICA group. These BMSC subpopulations remained unchanged or increased in the bone marrow, suggesting an effect of ICA on BMSC proliferation within the bone marrow. Activation of BMCSs and bone marrow cell transplantation has protective effects after ischemic brain damage [24]. However, to our knowledge the effect of ICA on the migration of mobilized BMSCs has not been reported. In the present study, a significant increase in the number of $CD45^+/CD34^+$ cells in the peripheral blood was observed. Among these bone marrow-derived progenitor cells, subpopulations additionally expressing the markers $CD31^+$ and c-kit were increased in the ICA-treated MCAO group. The observed increase in BMSCs was time-dependent, and reached a peak at seven days after stroke induction. Migration of BMSCs depends on homing factors, which mediate attraction, adhesion, and migration of these cells. SDF-1α, the ligand for CXCR-4, protein and mRNA levels were up-regulated compared to control. The mRNA and protein levels of CXCR-4, which is the corresponding surface receptor, also significantly increased in the ICA treatment group compared to controls. BMSCs can alleviate ischemic brain injury via the CXCR4/SDF-1α axis [25]. G-CSF is one of the most reliable BMSC mobilizing agents for tissue repair. However, it has poor chemotaxis properties for $CXCR4^+$ cells [10]. It is tempting to speculate that ICA might improve $CXCR4^+$ chemotaxis, improving tissue repair in ischemia after stroke.

The brain contains a reservoir of progenitor cells that may play a key role in the repair of ischemic brain damage [26]. To investigate these cells, we quantified $CD45^+/CD34^+/c\text{-}Kit^+$ cells. The results revealed a mild increase in $CD45^+/CD34^+/c\text{-}Kit^+$ cells both in the peripheral blood and in the bone marrow of MCAO rats. One other thing to note is that enhancement of BMSC homing may not be suitable for all stroke patients. MSCs were reported to be recruited and enriched in tumors, such as hepatocellular carcinoma, and MSCs may show trophic effects on tumors [27, 28]. Currently it is unclear whether MSCs have a positive or negative impact on tumor progression. Thus, it remains to be determined whether enhancing BMSC homing is an appropriate treatment strategy in stroke patients with malignant tumors.

A shortcoming of our study is that we only focused on investigating the effects of ICA on the SDF-1α/CXCR4 chemokine axis and on BMSC migration. In addition, we did not address a link between the SDF-1/CXR4 axis and BMSC mobilization, as well as the signaling pathways involved in regulating the SDF-1α/CXCR4 chemokine axis in the injured brain following ICA treatment.

In summary, our data demonstrate that in vivo administration of ICA can enhance BMSC homing and the expression of homing factors CXCR-4 and SDF-1α. Additional studies are needed to further investigate the effects of ICA and its mechanism of action. Stroke treatment with ICA and other agents, such as G-CSF, may optimize regeneration of an ischemic brain following a stroke.

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Disclosure of conflict of interest

None.

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References

[1] Maldonado NJ, Kazmi SO and Suarez JI. Update in the management of acute ischemic stroke. Crit Care Clin 2014; 30: 673-697.

[2] Li Y, Chen J, Chen XG, Wang L, Gautam SC, Xu YX, Katakowski M, Zhang LJ, Lu M, Janakiraman N, Chopp M. Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. Neurology 2002; 59: 514-523.

[3] Balseanu AT, Buga AM, Catalin B, Wagner DC, Boltze J, Zagrean AM, Reymann K, Schaebitz W and Popa-Wagner A. Multimodal approaches for regenerative stroke therapies: combination of granulocyte colony-stimulating factor with bone marrow mesenchymal stem cells is not superior to g-csf alone. Front Aging Neurosci 2014; 6: 130.

[4] Huang C, Gu H, Zhang W, Manukyan MC, Shou W and Wang M. SDF-1/CXCR4 mediates acute protection of cardiac function through myocardial stat3 signaling following global ischemia/reperfusion injury. Am J Physiol Heart Circ Physiol 2011; 301: H1496-1505.

[5] Lehwald N, Duhme C, Wildner M, Kuhn S, Furst G, Forbes SJ, Jonas S, Robson SC, Knoefel WT, Schmelzle M, Schulte Am Esch J. HGF and SDF-1-mediated mobilization of cd133+ bmsc for hepatic regeneration following extensive liver resection. Liver Int 2014; 34: 89-101.

[6] Klein RS, Rubin JB, Gibson HD, DeHaan EN, Alvarez-Hernandez X, Segal RA and Luster AD. SDF-1 alpha induces chemotaxis and enhances sonic hedgehog-induced proliferation of cerebellar granule cells. Development 2001; 128: 1971-1981.

[7] Kondziolka D, Wechsler L and Achim C. Neural transplantation for stroke. J Clin Neurosci 2002; 9: 225-230.

[8] Shyu WC, Lin SZ, Yen PS, Su CY, Chen DC, Wang HJ and Li H. Stromal cell-derived factor-1 alpha promotes neuroprotection, angiogenesis, and mobilization/homing of bone marrow-derived cells in stroke rats. J Pharmacol Exp Ther 2008; 324: 834-849.

[9] Lazarini F, Tham TN, Casanova P, Arenzana-Seisdedos F and Dubois-Dalcq M. Role of the alpha-chemokine stromal cell-derived factor (SDF-1) in the developing and mature central nervous system. Glia 2003; 42: 139-148.

[10] Zaruba MM and Franz WM. Role of the sdf-1-CXCR4 axis in stem cell-based therapies for ischemic cardiomyopathy. Expert Opin Biol Ther 2010; 10: 321-335.

[11] Liu H, Liu S, Li Y, Wang X, Xue W, Ge G and Luo X. The role of SDF-1-CXCR4/CXCR7 axis in the therapeutic effects of hypoxia-preconditioned mesenchymal stem cells for renal ischemia/reperfusion injury. PLoS One 2012; 7: e34608.

[12] Wang X, Li J, Qian L, Zang XF, Zhang SY, Wang XY, Jin JL, Zhu XL, Zhang XB, Wang ZY, Xu Y. Icariin promotes histone acetylation and attenuates post-stroke cognitive impairment in the central cholinergic circuits of mice. Neuroscience 2013; 236: 281-288.

[13] Guo J, Li F, Wu Q, Gong Q, Lu Y and Shi J. Protective effects of icariin on brain dysfunction induced by lipopolysaccharide in rats. Phytomedicine 2010; 17: 950-955.

[14] Jia J, Guan D, Zhu W, Alkayed NJ, Wang MM, Hua Z and Xu Y. Estrogen inhibits fas-mediated apoptosis in experimental stroke. Exp Neurol 2009; 215: 48-52.

[15] Xu RX, Wu Q, Luo Y, Gong QH, Yu LM, Huang XN, Sun AS and Shi JS. Protective effects of icariin on cognitive deficits induced by chronic cerebral hypoperfusion in rats. Clin Exp Pharmacol Physiol 2009; 36: 810-815.

[16] Benedek A, Móricz K, Jurányi Z, Gigler G, Lévay G, Hársing LG Jr, Mátyus P, Szénási G and Albert M. Use of ttc staining for the evaluation of tissue injury in the early phases of reperfusion after focal cerebral ischemia in rats. Brain Res 2006; 1116: 159-165.

[17] Yanamoto H, Hong SC, Soleau S, Kassell NF and Lee KS. Mild postischemic hypothermia limits cerebral injury following transient focal ischemia in rat neocortex. Brain Res 1996; 718: 207-211.

[18] Garcia JH, Wagner S, Liu KF and Hu XJ. Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats. Statistical validation. Stroke 1995; 26: 627-634.

[19] Jin Q, Lee C, Lee JW, Yeon ET, Lee D, Han SB, Hong JT, Kim Y, Lee MK and Hwang BY. 2-Phenoxychromones and prenylflavonoids from epimedium koreanum and their inhibitory effects on lps-induced nitric oxide and interleukin-1beta production. J Nat Prod 2014; 77: 1724-1728.

[20] Liao HJ, Chen XM and Li WG. [Effect of epimedium sagittatum on quality of life and cellular

immunity in patients of hemodialysis maintenance]. Zhongguo Zhong Xi Yi Jie He Za Zhi 1995; 15: 202-204.
[21] Yan S, Wu B, Lin Z, Jin H, Huang J, Yang Y, Zhang X, Shen Z and Zhang W. Metabonomic characterization of aging and investigation on the anti-aging effects of total flavones of epimedium. Mol Biosyst 2009; 5: 1204-1213.
[22] Wang J, Tian XF, Wu SY, Meng XC and Wen GW. Accelerated healing by composites containing herb epimedium for osteoinductive regeneration. Biomed Mater 2014; 9: 035013.
[23] Zhu HR, Wang ZY, Zhu XL, Wu XX, Li EG and Xu Y. Icariin protects against brain injury by enhancing sirt1-dependent pgc-1alpha expression in experimental stroke. Neuropharmacology 2010; 59: 70-76.
[24] Lin MN, Shang DS, Sun W, Li B, Xu X, Fang WG, Zhao WD, Cao L and Chen YH. Involvement of pi3k and rock signaling pathways in migration of bone marrow-derived mesenchymal stem cells through human brain microvascular endothelial cell monolayers. Brain Res 2013; 1513: 1-8.
[25] Schonemeier B, Schulz S, Hoellt V and Stumm R. Enhanced expression of the CXCl12/SDF-1 chemokine receptor CXCR7 after cerebral ischemia in the rat brain. J Neuroimmunol 2008; 198: 39-45.
[26] Zhang SC and Fedoroff S. Expression of stem cell factor and c-kit receptor in neural cells after brain injury. Acta Neuropathol 1999; 97: 393-398.
[27] Hernanda PY, Pedroza-Gonzalez A, van der Laan LJ, Broker ME, Hoogduijn MJ, Ijzermans JN, Bruno MJ, Janssen HL, Peppelenbosch MP and Pan Q. Tumor promotion through the mesenchymal stem cell compartment in human hepatocellular carcinoma. Carcinogenesis 2013; 34: 2330-2340.
[28] Hernanda PY, Pedroza-Gonzalez A, Sprengers D, Peppelenbosch MP and Pan Q. Multipotent mesenchymal stromal cells in liver cancer: implications for tumor biology and therapy. Biochim Biophys Acta 2014; 1846: 439-445.