European Review for Medical and Pharmacological Sciences 2017; 21: 3916-3923

Facilitation of ultrasonic microvesicles on homing and molecular mechanism of bone marrow mesenchymal stem cells in cerebral infarction patients

F. CHANG, W. XIONG, D. WANG, X.-Z. LIU, W. ZHANG, M. ZHANG, P. JING

Department of Neurology, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China

Abstract. – OBJECTIVE: Cerebral infarction, or ischemia brain stroke, is a common cerebrovascular disease. Bone marrow mesenchymal stem cells (BMSCs) are widely used to treating ischemia disease such as cardiac infarction. Ultrasonic microvesicles may help the targeting of exogenous factors via localized energy blast. Therefore, this study aims to investigate the effect of ultrasonic microvesicles on the homing of BMSCs on artery thrombosis and the associated molecular mechanisms.

MATERIALS AND METHODS: Rat BMSCs were isolated and cultured. Rats were divided into sham, model, BMSCs, and microvesicles groups. Cerebral infarction model was prepared by ligation of cervical artery and middle cerebral artery. 3×106/kg BMSCs were transplanted via tail veins. Microvesicles were used for assisting BMSCs infusion. Sex-determining region Y (SRY) gene expression was measured by Real-time PCR, while 2,3,5-triphenyltetrazolium chloride (TTC) staining was employed for describing the area of cerebral infarction. The activity of caspase 3 was assayed by test kit. Vascular endothelial growth factor (VEGF), nuclear factor kappa B (NF-κB) mRNA/protein levels, were quantified by Real-time PCR, and Western blotting, respectively.

RESULTS: Microvesicle group had significantly elevated SRY expression ($p<0.05$ compared to BMSCs group). Transplantation of BMSCs remarkably decreased cerebral infarction area, caspase 3 activity or NF-κB expression, and increased VEGF expression ($p<0.05$ compared to model group). Microvesicle induced BMSCs had more potent effects ($p<0.01$).

CONCLUSIONS: Ultrasound microvesicle facilitated homing of BMSCs in cerebral infarction, and improved infarction disease via up-regulating VEGF expression, inhibiting NF-κB expression, and modulating apoptosis.

Key Words:
Cerebral infarction, Ultrasonic microvesicle, Bone marrow mesenchymal stem cells, Homing, VEGF.

Introduction

Cerebral infarction, or ischemia brain stroke, is a common cerebrovascular disease[1,2]. Various risk factors exist for cerebral infarction, including coronary heart disease, hypertension, elevated body mass index (BMI), diabetes mellitus and hyperlipemia, or family history[3]. With transition of life styles and diet habits, plus working stress and environmental factors, incidence of cerebral infarction is rapidly increasing by years[4]. Cerebral infarction can occur at all age groups, with highest frequency in 45-60 years people[5]. Atherosclerosis of cerebral vessels can cause injury of vascular endothelial, leading to stenosis of cerebral artery cavity. Multiple factors can aggravate cerebral infarction for focal artery thrombosis and stenosis of artery or even full blockade, leading to brain tissue hypoxia, ischemia or necrosis, further causing neural injury and brain dysfunction[6]. Previous studies[7,8] showed decreased expression of vascular endothelial growth factor (VEGF) and activation of nuclear factor kappa B (NF-κB). Due to the severe effects on patient life quality, the treatment of cerebral ischemia has drawn lots of interests by world health organization (WHO)[9]. However, no satisfactory treatment efficacy has been received for cerebral infarction.

Bone marrow mesenchymal stem cells (BMSCs) has features including highly self-renewal, self-differentiation and minor immunogenicity, and have been widely applied in treating ischemia diseases including cardiac infarction[10,11]. BMSCs can differentiate toward endothelial cells, which further facilitate *de novo* angiogenesis for tissue repair, and are thus optimal stem cells for treating ischemia diseases[12,13]. Previous methods using intravenous infusion of BMSCs had high safety and are easy for ma-

Corresponding Author: Wei Xiong, MD; e-mail: WeiXiongzxc@163.com

nipulation. However, lower specificity and efficiency exists[14], thus making the improvement of transplantation efficiency a critical step of obtaining better outcomes. Sex-determining region Y (SRY) gene has one typical DNA binding domain and can code for transcriptional factor. SRY gene has been widely applied to test BMSCs contents[15]. With the advancement of ultrasound contrast agent, ultrasound microvesicles technique can work as gene vector or drug delivery vehicle in addition to ultrasound imaging, and it has been demonstrated to have high efficiency, stability and safety[16]. Ultrasound microvesicles can locate precisely and benefited the transportation of exogenous substances to targeted sites via ultrasound energy blast[17]. This study aimed to investigate the induction of ultrasound microvesicles on the homing of artery thrombosis in cerebral infarction and possible molecular mechanisms.

Materials and Methods

Experimental Animal

Healthy male Sprague-Dawly (SD) rats (2 month age, specific pathogen free (SPF) grade, body weight 250±20 g) were purchased in the laboratory animal center of Wuhan University and were kept in an SPF grade facility. Animals were kept under fixed temperature (21±1°C) and relative humidity conditions (50-70%) with 12 h/12 h light/dark cycle.

Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of the Central Hospital of Wuhan, Wuhan, China.

Materials and Equipment

SF6-microvesicles were purchased from Bracco Suisse S.A. (Plan-les-Ouates Geneve, Switzerland). Sodium pentobarbital was purchased from Zhaohui Pharma. Co. Ltd. (Shanghai, China). Polyvinylidene fluoride (PVDF) membrane was provided by Pall Life Sciences (Covina, CA, USA). Chemical reagents for Western blotting and 2,3,5-triphenyltetrazolium chloride (TTC) powders were purchased from Beyotime Biotech. (Shanghai, China). Enhanced chemiluminescence (ECL) reagent was purchased from Amersham Biosciences (Little Chalfont, Buckinghamshire, UK). Rabbit anti-mouse VEGF monoclonal antibody, rabbit anti-mouse NF-κB monoclonal antibody and goat anti-rabbit horseradish peroxidase (HRP) labeled IgG secondary antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Caspase 3 activity kit was produced by Cell Signaling Technology Inc. (Beverly, MA, USA). RNA extraction and reverse transcription kit were purchased from Axygen (Tewksbury, MA, USA). ABI7900HT Real-time PCR cycler was purchased from ABI (Foster City, CA, USA). Surgical microscope was purchased from Suzhou Medical Instrument (Suzhou, China). Microplate reader was purchased BD (San Jose, CA, USA). Other common reagents were purchased from Sangon Biotech. Co. Ltd. (Shanghai, China).

Isolation, Culture and Identification of rat BMSCs

One healthy SD rat was sacrificed. After sterilization by 70% ethanol immersion, femur and tibia were exposed to remove epiphysis and attached soft tissues at terminus. Isolated femur and tibia were placed into sterile dish. 3 ml bone marrow was collected by syringe containing 2 ml heparin, and was placed into 10 ml serum-free culture medium. A total of 3 ml phosphate buffer solution (PBS) was then added to prepare cell suspension. Bone marrow was placed in 10 ml centrifuge tube for 1000 r/min centrifugation for 10 min. Supernatant and lipid were discarded, followed by 2500 r/min centrifugation for 25 min. A total of 5 ml BMSC suspensions were slowly applied onto the surface of monocyte separation buffer, for 2000 r/min centrifugation for 20 min. The white matter in the middle layer was saved and transferred to another tube, which was centrifuged at 1000 r/min for 5 min, followed by two times of rinsing. Bone marrow tissues were then placed into 5 ml low glucose Dulbecco's modified medium (L-DMEM) containing 10% fetal bovine serum (FBS) and 1% dual-antibiotics. Cells were cultured in a 37°C chamber with 5% CO_2 for 48 h with changing medium. Non-attached cells were removed for continuous culture, with changing medium every 3 days. Cells were passed when reaching 80% confluence. 1×10^5/ml BMSCs were inoculated into 24-well plate containing 500 µl L-DMEM. Cells were identified when reaching 80% confluence. After discarding culture medium cells were rinsed in PBS, and were fixed in 4% paraformaldehyde. With PBS rinsing, 300 µl specific markers (CD29, CD44, CD34, CD45 monoclonal antibody, at 1:100 dilution) were added for 4°C overnight incubation. On the next day, PBS was used to

rinse away primary antibody, followed by the addition of goat anti-rabbit FITC labeled-secondary antibody for room temperature incubation. Secondary antibody was washed away for observation under microscope.

Grouping of Experimental Animals

Rats were randomly divided into four groups: including sham group, model group (which was prepared for cerebral infarction model using ligation of carotid artery and middle cerebral artery), BMSCs group (which received transplantation of 3×10^6/kg BMSCs by tail veins) and microvesicle group (which received 3×10^6/kg BMSCs by ultrasound microvesicles).

Preparation of Rat Cerebral Model and Treatment

Rats were anesthetized by intraperitoneal injection of 30 mg/kg sodium pentobarbital, and were fixed on the plate in supine position. As previously recorded[18], an incision was made 2-2.5 cm left of middle line to completely expose the triangle area between sternohyoid muscle and sternocleidomastoid muscle, making common carotid artery and exterior carotid artery visible. Exterior carotid artery was ligated by surgical silks, while common carotid artery was clipped. One small hole was punctured using 1 ml syringe needle, followed by the insertion of 0.2 mm diameter fishing line. The artery clip was released to free the fishing line, which was stopped and blocked the middle cerebral artery, whose flood supply was blocked. Common carotid artery and puncture incision were sutured by layers. Penicillin (2×10^4 U/kg) was intramuscularly injected for disinfection. In sham group, common carotid artery and external carotid artery were isolated but not ligated. In BMSCs group, 3×10^6/kg BMSCs were transplanted via tail veins after preparing models. In microvesicle group, 0.1 ml microvesicle contrast agent was infused via tail vein, followed by ultrasound on brain, and 3×10^6/kg BMSCs transplantation via tail vein.

Evaluation of Cerebral Infarction Rate

Whole brain was isolated from all groups of rats, and was removed for cerebellum and olfactory bulb, and lower brain stem. The 2 mm brain sections were prepared, and were incubated in dark using 0.4% TCC solution in dark at 37°C for 20 min. Post-stain brain sections were fixed in 10% formalin for 2 min. Infarction area percentage was analyzed by imaging processing software (Bio-Rad Laboratories, Hercules, CA, USA) in all groups.

Caspase 3 Activity Assay

Caspase 3 activity was evaluated using test kit. In brief, cells were digested by trypsin, and were centrifuged at 600 ×g for 5 min under 4°C. The supernatant was discarded, followed by the addition of cell lysis buffer and iced incubation for 15 min. The mixture was then centrifuged at 20000×g for 5 min under 4°C, followed by the addition of 2 mM Ac-DECD-pNA. Optical density (OD) values at 450 nm wavelength were measured to reflect caspase 3 activity.

Real Time PCR for SRY, VEGF and NF-κB mRNA Expression in Brain Tissues

Trizol reagent was used to extract total RNA from rat brain tissues. RNA purity and concentration were determined by ultraviolet-visible (UV) spectrometry. Reverse transcription was performed using test kit. The complementary DNA (cDNA) was synthesized by specific primers designed from PrimerPremier 6.0. Real-time PCR was used to test target gene expression using specific primers (Table I) synthesized by Sangon Biotech. Co. Ltd. (Shanghai, China). Real-time PCR was performed under the following conditions: 52°C 1 min, followed by 35 cycles each containing 90°C for 30 s, 58°C for 50 s and 72°C for 35 s. Fluorescent quantitative PCR was used to collect all data. The cycle threshold (CT) values were calculated using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene as the internal reference to plot a standard curve, on which quantitative analysis was performed by $2^{-\Delta Ct}$ method.

Table I. Primer synthesis sequences.

Target gene	Forward primer 5′-3′	Reverse primer 5′-3′
GADPH	AGTGCCAGCCTCGTCTCATAG	CGTTGAACTTGCCGTGGGTAG
SRY	GACTAGGAATACGAACCGCTT	GCCTCGATCCTCCGAGATT
VEGF	TGAACAATCTAGATCACGGCG	GGGACTCTCCGTCATCTAC
NF-κB	CTACTGCCGTCCGATTGAG	GCTCATCTCTCCTATGTGCTGG

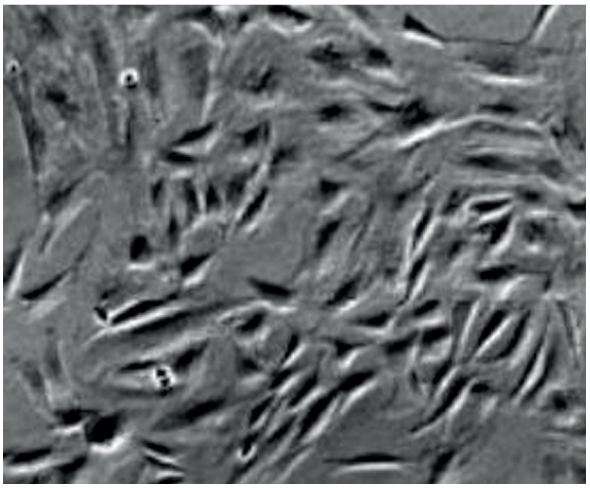

Figure 1. Morphology of BMSCs after 3rd passage (×100).

Western Blotting for VEGF and NF-κB Protein Expression

Brain tissues were added with lysis buffer on ice for 15-30 min, followed by ultrasound rupture (5 s × 4) and centrifugation (4°C, 10000×g, 15 min). Supernatants were saved and quantified for protein contents, and were stored at -20°C for further Western blotting. Proteins were then separated using 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and were transferred to polyvinylidene fluoride (PVDF) membrane using semi-dry method (160 mA for 1.5 h). Non-specific background was removed by 5% defatted milk powder at room temperature for 2 h, followed by the addition of anti-VEGF monoclonal antibody (1:1000 dilution) and anti-NF-κB monoclonal antibody (1:2000) in 4°C overnight incubation. On the next day, the membrane was rinsed in PBS tween 20 (PBST), and incubated with goat anti-rabbit secondary antibody (1:2000) for 30 min incubation. After PBST rinsing, ECL reagent was used to develop the membrane for 1 min, followed by exposure under X-ray for observing results. Protein imaging analysis system and Quantity One software were used to scan X-ray films for observing band density. Each experiment was repeated for four times (n = 4) for statistical analysis.

Statistical Analysis

SPSS19.0 software (SPSS Inc., Chicago, IL, USA) was used to analyze all data, of which measurement data were presented as mean ± standard deviation (SD). For the One way analysis of variance (ANOVA), the LSD Post-hoc test was used to compare means across multiple groups. A statistical significance was defined when $p<0.05$.

Results

Morphology Observation and Identification of BMSCs

Primary cultured rat BMSCs were inoculated. We found large amounts of suspended round cells and debris. Further observation of primary cultured cells at 24 h found attached growth of cells, with smaller spindle shapes and perturbation, which was elongated with further culture. Cells showed colony growth with more perturbations, and gradually transformed to evenly distributed proliferation. Cells were transformed to narrowly arrange large spindle shapes with squirrel like features from short spindle. After passage, cells showed similar morphology (Figure 1). Immunofluorescent assay showed positive expression for CD29 and CD44 of BMSCs (Figure 2).

Facilitation of BMSCs Homing by Ultrasound Microvesicle

Real-time PCR was used to test SRY mRNA expression in BMSCs and ultrasound microvesicle groups, for determining the effect of ultrasound microvesicle on homing of BMSCs. Results showed significantly elevated SRY mRNA expression by ultrasound microvesicle ($p<0.05$ compared to BMSCs group, Figure 3).

Facilitation of BMSCs Homing by Ultrasound Microvesicle and Effects on Cerebral Infarction

We measured the effect on rat cerebral infarction area after facilitating BMSCs homing by ultrasound microvesicle. Results showed significantly elevated infarction area in rats ($p<0.05$ compared to sham group). The facilitation of homing by tail vein injection of BMSCs or ultrasound microvesicle all significantly increased cerebral infarction area percentage ($p<0.05$ compared to model group). Ultrasound microvesicle group had more potent suppression of cerebral infarction area ($p<0.01$, Figure 4).

Effects on Caspase 3 activity by the Facilitation of BMSCs Homing from Ultrasound Microvesicle

Caspase 3 activity assay kit was used to analyze the effect of ultrasound microvesicle-facilitated BMSCs homing on caspase 3 activity on cerebral

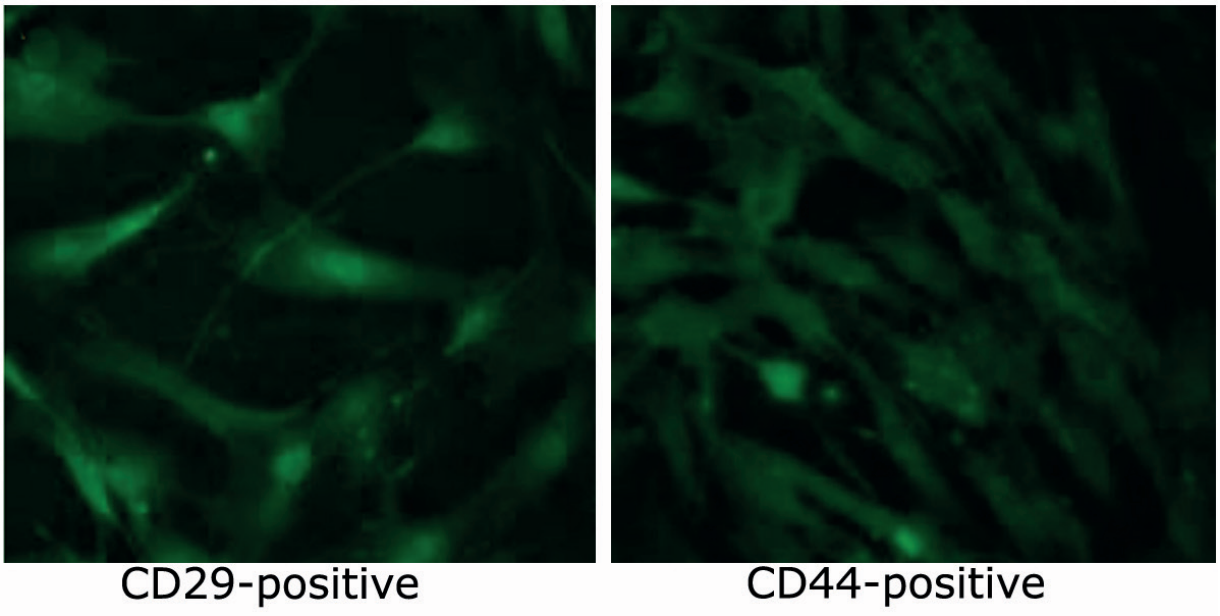

Figure 2. Characterization of BMSCs (×100).

infarction rats. Results showed significantly elevated caspase 3 activities in rat brain tissues in cerebral infarction model group (p<0.05 compared to sham group). The facilitation of homing by tail vein injection of BMSCs or ultrasound microvesicle all significantly lowered caspase 3 activity (p<0.05 compared to model group). Ultrasound microvesicle group had more potent effects (p<0.01, Figure 5).

Effects on VEGF and NF-κB mRNA by the facilitation of BMSCs Homing from Ultrasound Microvesicle

Real-time PCR was used to analyze the effect of ultrasound microvesicle-facilitated BMSCs homing on VEGF and NF-κB mRNA expression in cerebral infarction rats. Results showed significantly decreased VEGF mRNA and increased NF-κB mRNA in rat brain tissues in cerebral infarction model group (p<0.05 compared to sham group). The facilitation of homing by tail vein injection of BMSCs or ultrasound microvesicle all significantly enhanced VEGF mRNA expression and lowered NF-κB mRNA expression (p<0.05 compared to model group). Ultrasound microvesicle group had more potent effects (p<0.01, Figure 6).

Effects on VEGF and NF-κB Protein Expression by the Facilitation of BMSCs Homing from Ultrasound Microvesicle

Western blotting was used to analyze the effect of ultrasound microvesicle-facilitated BMSCs homing on VEGF and NF-κB protein expression

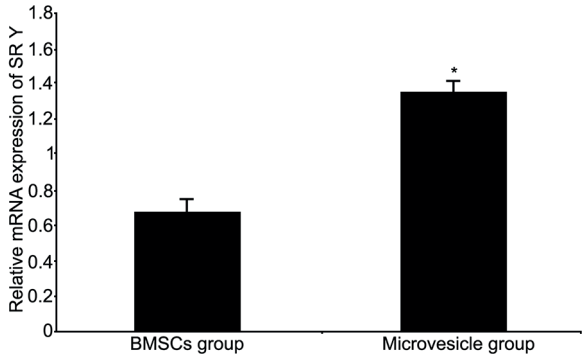

Figure 3. SRY mRNA expression analysis in BMSCs. *, p<0.05 compared to BMSCs group.

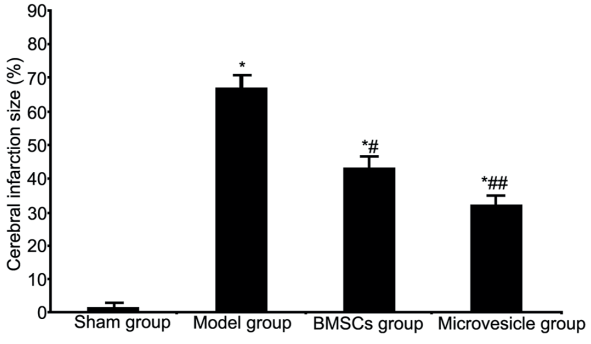

Figure 4. Facilitation of BMSCs homing by ultrasound microvesicle and effects on cerebral infarction. *, p<0.05 compared to sham group; #, p<0.05, ##, p<0.01 compared to model group.

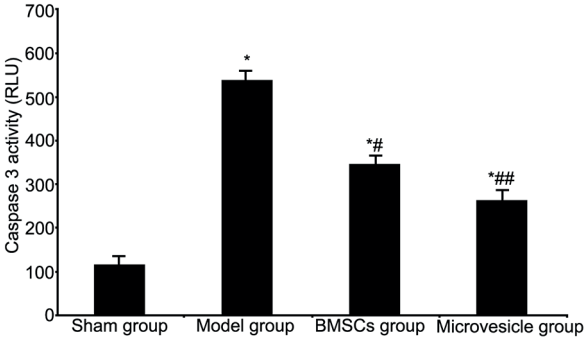

Figure 5. Effects on caspase 3 activity by the facilitation of BMSCs homing from ultrasound microvesicle. *, $p<0.05$ compared to sham group; #, $p<0.05$, ##, $p<0.01$ compared to model group.

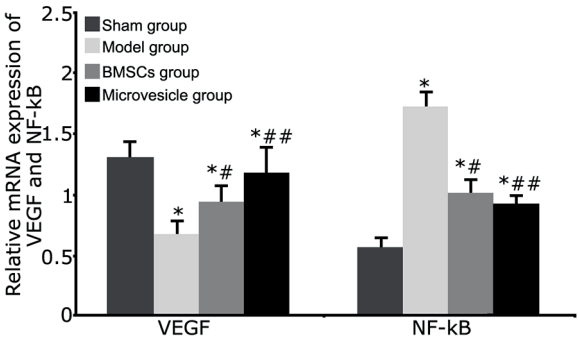

Figure 6. Effects on VEGF and NF-κB mRNA by the facilitation of BMSCs homing from ultrasound microvesicle. *, $p<0.05$ compared to sham group; #, $p<0.05$, ##, $p<0.01$ compared to model group.

in cerebral infarction rats. Results showed significantly decreased VEGF and increased NF-κB protein in rat brain tissues in cerebral infarction model group ($p<0.05$ compared to sham group). The facilitation of homing by tail vein injection of BMSCs or ultrasound microvesicle all significantly enhanced VEGF expression and lowered NF-κB protein expression ($p<0.05$ compared to model group). Ultrasound microvesicle group had more potent effects ($p<0.01$, Figures 7 and 8).

Discussion

Focal blood supply dysfunction of brain caused by various reasons can lead to cerebral ischemia-hypoxia lesion and necrosis, further leading to relevant neurological disorder in clinics. Among all subtypes of brain infarction, cerebral artery thrombosis is the most common one[19]. As ischemia brain injury is often accompanied with vascular endothelial injury, neural cell injury or dysfunction, the effective facilitation on endothelial cell regeneration and treating neurological injury are critical questions in treating brain infarction[20]. BMSCs as pluripotent stem cells type, have pluripotent differentiation potency, including those towards neural cells and endothelial tissues, thus making them one novel type of target cells for brain ischemia treatment[21]. Previous study[22] showed the repair of injury site via venous transplantation of BMSC, indicating a homing function by BMSC via migration inside the body, although leaving its molecular mechanism not being illustrated.

This study isolated and cultured rat BMSCs, on which morphology observation and immunofluorescent identification were performed. Results showed fibroblast-like morphology of BMSCs, which had parallel arrangement of elongated spindle shapes. Immunofluorescence revealed positive expression for CD29 and CD44 of BMSCs, indicating BMSCs features of isolated cells in this work. As venous transfusion of BMSCs had unfavorable targeting and lower efficiency, further ultrasound

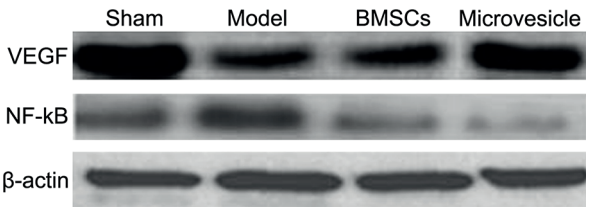

Figure 7. Effects on VEGF and NF-κB protein expression by the facilitation of BMSCs homing from ultrasound microvesicle.

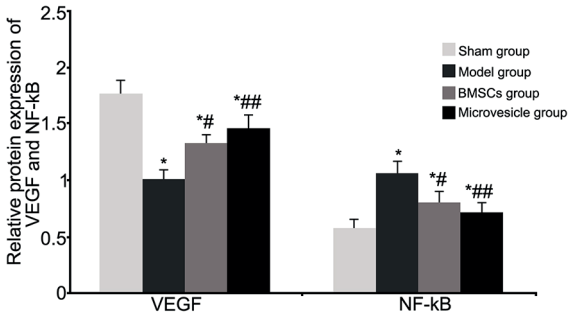

Figure 8. Analysis of effects on VEGF and NF-κB protein expression by the facilitation of BMSCs homing from ultrasound microvesicle. *, $p<0.05$ compared to sham group; #, $p<0.05$, ##, $p<0.01$ compared to model group.

microvesicles could facilitate their homing. Ultrasound microvesicles can be targeted to specific sites, and interact with peripheral tissues and cells via cavitation and mechanical effects, to elevate permeability of vessels and membrane, plus the providing for suitable environment for survival of migrated stem cells[23]. This study utilized SRY gene to track BMSCs expression, and found significantly elevated BMSCs homing in cerebral infarction, and improvement of cerebral infarction area for improving infarction condition.

Further analysis of related molecular mechanism revealed large amounts of neuron apoptosis or death after cerebral ischemia injury, accompanied with significantly elevated caspase 3 activity. Further injury of vascular endothelial cells can decrease VEGF secretion and down-expression[24]. In the formation of cerebral infarction, NF-κB activation, elevated expression can up-regulate the expression of multiple pro-inflammatory factors, adhesion molecule and chemokines to form an inflammatory network, further aggravating cerebral infarction[7,25]. This paper demonstrated significantly depressed cerebral infarction area after BMSCs transplantation, plus lower caspase 3 activity or NF-κB expression, and elevated VEGF expression. Microvesicles induced BMSCs transplantation had more potent effect. These results collectively suggested that BMSC transplantation could improve cell proliferation-apoptotic balance in injury brain tissues, while ultrasound microvesicles could facilitate BMSC homing, further improving proliferation-apoptosis balance, suppressed inflammatory environment, and up-regulated VEGF expression, further facilitating repair of vascular endothelial cells.

Conclusions

Ultrasound microvesicles could facilitate homing of cerebral infarction, and improved cerebral infarction via up-regulating VEGF expression, inhibiting NF-κB expression and regulating apoptosis.

Conflict of interest

The authors declare no conflicts of interest.

References

1) He X, Li DR, Cui C, Wen LJ. Clinical significance of serum MCP-1 and VE-cadherin levels in patients with acute cerebral infarction. Eur Rev Med Pharmacol Sci 2017; 21: 804-808.
2) Shen B, Liu Q, Gu Y, Wang Y, Zhang Z. Efficacy and safety evaluation on arterial thrombolysis in treating acute cerebral infarction. Cell Biochem Biophys 2015; 73: 297-304.
3) Ma XM, Liu M, Liu YY, Ma LL, Jiang Y, Chen XH. Ischemic preconditioning protects against ischemic brain injury. Neural Regen Res 2016; 11: 765-770.
4) Kotlega D, Golab-Janowska M, Masztalewicz M, Ciecwiez S, Nowacki P. The emotional stress and risk of ischemic stroke. Neurol Neurochir Pol 2016; 50: 265-270.
5) Ma LL, Shong L, Yu XD, Yu TK, Liang H, Qiu JX. The clinical study on the treatment for acute cerebral infarction by intra-arterial thrombolysis combined with mild hypothermia. Eur Rev Med Pharmacol Sci 2017; 21: 1999-2006.
6) Petrovic-Djergovic D, Goonewardena SN, Pinsky DJ. Inflammatory disequilibrium in stroke. Circ Res 2016; 119: 142-158.
7) Zuo L, Shi L, Yan F. The reciprocal interaction of sympathetic nervous system and cAMP-PKA-NF-kB pathway in immune suppression after experimental stroke. Neurosci Lett 2016; 627: 205-210.
8) Shimizu T, Dietz RM, Cruz-Torres I, Strnad F, Garske AK, Moreno M, Venna VR, Quillinan N, Herson PS. Extended therapeutic window of a novel peptide inhibitor of TRPM2 channels following focal cerebral ischemia. Exp Neurol 2016; 283: 151-156.
9) Colantonio LD, Gamboa CM, Kleindorfer DO, Carson AP, Howard VJ, Muntner P, Cushman M, Howard G, Safford M. Stroke symptoms and risk for incident coronary heart disease in the REasons for Geographic And Racial Differences in Stroke (REGARDS) study. Int J Cardiol 2016; 220: 122-128.
10) Isbambetov A, Baimakhanov Z, Soyama A, Hidaka M, Sakai Y, Takatsuki M, Kuroki T, Eguchi S. Equal distribution of mesenchymal stem cells after hepatic ischemia-reperfusion injury. J Surg Res 2016; 203: 360-367.
11) Li C, Wei GJ, Xu L, Rong JS, Tao SQ, Wang YS. The involvement of senescence induced by the telomere shortness in the decline of osteogenic differentiation in BMSCs. Eur Rev Med Pharmacol Sci 2017; 21: 1117-1124.
12) Teng ZW, Zhu Y, Na Q, Zhang XG, Zhao H, Wei GJ, Zhao J, Zhang XW, Wang GZ, Liang J, Zhang ZJ, Li YG. Regulatory effect of miRNA on multi-directional differentiation ability of mesenchymal stem cell in treatment of osteoporosis. J Biol Regul Homeost Agents 2016; 30: 345-352.
13) Cavallo C, Desando G, Ferrari A, Zini N, Mariani E, Grigolo B. Hyaluronan scaffold supports osteogenic differentiation of bone marrow concentrate cells. J Biol Regul Homeost Agents 2016; 30: 409-420.
14) Wu JH, Li M, Liang Y, Lu T, Duan CY. Migration of adipose-derived mesenchymal stem cells stably expressing chondroitinase ABC In vitro. Chin Med J (Engl) 2016; 129: 1592-1599.

15) Ding Z, Liu X, Ren X, Zhang Q, Zhang T, Qian Q, Liu W, Jiang C. Galectin-1-induced skeletal muscle cell differentiation of mesenchymal stem cells seeded on an acellular dermal matrix improves injured anal sphincter. Discov Med 2016; 21: 331-340.

16) Ren ST, Shen S, He XY, Liao YR, Sun PF, Wang B, Zhao WB, Han SP, Wang YL, Tian T. The effect of docetaxel-loaded micro-bubbles combined with low-frequency ultrasound in H22 hepatocellular carcinoma-bearing mice. Ultrasound Med Biol 2016; 42: 549-560.

17) Tzanakis I, Xu WW, Eskin DG, Lee PD, Kotsovinos N. In situ observation and analysis of ultrasonic capillary effect in molten aluminium. Ultrason Sonochem 2015; 27: 72-80.

18) Moon JH, Na JY, Lee MC, Choi KH, Lee JK, Min JJ, Kim KT, Park JT, Park MS, Kim HS. Neuroprotective effects of systemic cerebral endothelial cell transplantation in a rat model of cerebral ischemia. Am J Transl Res 2016; 8: 2343-2353.

19) Sugidachi A, Mizuno M, Ohno K, Jakubowski JA, Tomizawa A. The active metabolite of prasugrel, R-138727, improves cerebral blood flow and reduces cerebral infarction and neurologic deficits in a non-human primate model of acute ischaemic stroke. Eur J Pharmacol 2016; 788: 132-139.

20) Altun I, Unal Y, Basaran O, Akin F, Emir GK, Kutlu G, Biteker M. Increased epicardial fat thickness correlates with aortic stiffness and N-terminal pro-brain natriuretic peptide levels in acute ischemic stroke patients. Tex Heart Inst J 2016; 43: 220-226.

21) Mun JY, Shin KK, Kwon O, Lim YT, Oh DB. Minicircle microporation-based non-viral gene delivery improved the targeting of mesenchymal stem cells to an injury site. Biomaterials 2016; 101: 310-320.

22) Zhang Y, Yang J, Zhang P, Liu T, Xu J, Fan Z, Shen Y, Li W, Zhang H. Calcitonin gene-related peptide is a key factor in the homing of transplanted human MSCs to sites of spinal cord injury. Sci Rep 2016; 6: 27724.

23) Sajjadi B, Abdul Aziz AR, Ibrahim S. Mechanistic analysis of cavitation assisted transesterification on biodiesel characteristics. Ultrason Sonochem 2015; 22: 463-473.

24) Cairos C, Schneider J, Pflieger R, Mettin R. Effects of argon sparging rate, ultrasonic power, and frequency on multibubble sonoluminescence spectra and bubble dynamics in NaCl aqueous solutions. Ultrason Sonochem 2014; 21: 2044-2051.

25) Mateos L, Perez-Alvarez MJ, Wandosell F. Angiotensin II type-2 receptor stimulation induces neuronal VEGF synthesis after cerebral ischemia. Biochim Biophys Acta 2016; 1862: 1297-1308.