Chapter 12
Combination Treatment of Mesenchymal Stem Cells (MSCs) and Angelica sinensis' Active Ingredients for Ischemic Stroke

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Abstract At present, mesenchymal stem cells (MSCs) are regarded as a candidate for neovascularization and tissue regeneration after ischemic stroke. Numerous studies reported that Angelica (also called Dong quai, a well-known Chinese herbal medicine) extracts and its active ingredients such as ligustilide, n-Butylphthalide and sodium ferulate had significant effects of anti-inflammatory, anti-activation of oxygen free radicals, angiogenesis, anti-platelet aggregation, neuroprotection and so on. Angelica' active compositions facilitated MSCs to migrate into infarcted zone and differentiation. Moreover, MSCs combined with angelica' active components improved neurological function and decreased infarcted volume, advanced neovascularization and neurogenesis, regulated astrocytes characteristics, enhanced regional cerebral blood flow and glucose metabolism, as well as reduced brain-blood barrier permeability in infarction. Consequently, the structure and function of neurovascular unit in infarct region partly obtained recovery. Therefore, the combination treatment was a valuable therapy aimed at improving post-stroke restoration.

Keywords Angelica sinensis • Combination treatment • Ischemic stroke • Mesenchymal stem cells

Abbreviations

BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
BMP	Bone morphogenetic proteins
BP	n-Butylidenephthalide

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BrdU	5-Bromo-2′-deoxyuridine
CBF	Cerebral blood flow
CXCR4	Chemokine (CXC motif) receptor-4
DCX	Doublecortin
DG	Dentate gyrus
EC	Endothelial cell
ERK	Extracellular signal-regulated kinases
FA	Ferulic acid
FDA	Food and Drug Administration
FDG	^{18}F-2-deoxy-glucose
GDNF	Glial cell line-derived neurotrophic factor
GFAP	Glial fibrillary acidic protein
HIF	Hypoxia-inducible factors
HUVEC	Human umbilical vein endothelial cell
MCAo	Middle cerebral artery occlusion
MRI	Magnetic resonance imaging
MSC	Mesenchymal stem cell
mTOR	Mammalian target of rapamycin
NBP	n-Butylphthalide
PET/CT	Positron emission tomography–computed tomography
PWI	Perfusion-weighted imaging
SDF-1	Stromal cell-derived factor-1
SF	Sodium ferulate
STEPS	Stem Cells as an Emerging Paradigm in Stroke
SVZ	Subventricular zone
TTC	2,3,5-Triphenyltetrazolium chloride
Tuj-1	Neuron-specific class III beta-tubulin
VEGF	Vascular endothelial growth factor
vWF	Von Willebrand factor

1 Introduction

Ischemic stroke is the most common cerebralvascular disease. Due to blood flow blockage by arterial thrombus, amounts of neurons in ischemic central and penumbral regions occur to necrosis and apoptosis, which resulted in attenuation of neurological function. Evidence indicated that stroke was the second leading cause of death and the major cause of disability globally, especially in developing countries [1]. There are 15 million individuals suffer from stroke in every year worldwide, and in the United States, among 800,000 stroke patients, 75% of them have never experienced stroke before, and 25% undergo recurrent attack [2]. Moreover, mortality of ischemic stroke is predicted to nearly double by 2032 [3]. Although stroke has been major threat to life expectancy and quality, there are relatively few treatment

options available to ameliorate neurological function due to complicated etiological and pathophysiological evolutions after ischemic stroke [4]. At present, recombinant tissue plasminogen activator (rt-PA) is still approved by United States Food and Drug Administration (FDA) for dissolution of thrombus and improvement of cerebral flow, but narrow therapeutic time window (3–6 h) and multiplicative individual exclusion criteria limit it to be widely applied [5, 6].

In recent years, cell therapy is regarded as a promising approach. Bone marrow-derived mesenchymal stem cells (MSCs) have been demonstrated to be able to differentiate into neuronal cells and replace injured neurons after cerebral ischemia, as well as activate endogenous restorative responses (e.g. neurogenesis, angiogenesis and synaptogenesis) against injured brain, so autologous MSCs were transplanted into stroke patients in 2005 [7–11]. Evidence indicated that five stroke patients accepted stereotactically transplanted MSCs treatment, and the therapeutic results showed that their neurological functions were improved and no complications happened after 1 year's observation [12]. In 2007, the National Institutes of Health and FDA issued consensus-based guidelines on the development of cell therapies for stroke, entitled "Stem Cells as an Emerging Paradigm in Stroke" (STEPS). Current STEPS 3 had discussed how to successful complete translation from animal models to patients and optimize clinical trial designs for acute and chronic stroke [13].

Angelica (Angelica sinensis (Oliv.) Diels), commonly called Dong quai in Chinese, is a dried root derived from an herb in the family Apiaceae, which is used over thousands of years as a well-known Chinese medicine. Since 1980s, angelica extract and its active ingredients began to be used to treat ischemic cerebrovascular disease. Liu and colleagues observed 1404 patients of acute cerebral infarction, and 692 of them treated with angelica injection, 390 of them used Danshen (Salvia miltiorrhiza) injection, 322 of them treated with low molecular dextran. Consequently, the total effective rate was 78.7%, 63.6% and 59.3% respectively after treatment, suggesting neurological functional recovery in angelica injection group was better than those in other two groups [14]. Study indicated that Z-Ligustilide, a main component of volatile oil in angelica, could reduce infarct volume and cerebral edema in a dose-dependent way, and ameliorate injured neurological function after 2 h in middle cerebral artery occlusion (MCAo) rats, suggesting it had obvious neuroprotective effect [15]. n-Butylphthalide (NBP) derived from phthalides compounds in angelica has been identified as a new drug for the treatment of ischemic cerebrovascular disease by China FDA. Previous study indicated that it could improve cognitive deficits in rats with chronic cerebral ischemia [16]. Moreover, in a randomized, double-blind and multi-center research on 535 stroke patients, it showed that patients' neurological functional scores in NBP group were obviously higher than those in ozagrel group [17]. The extract and active ingredients in angelica exert multi-efficacy in the treatment of ischemic stroke.

It has been demonstrated that the effect of combining MSCs with pharmacological agents on stroke is superior to MSCs treatment alone or pharmacological agents. For example, combined treatment of BMSCs with simvastatin could further facilitate MSCs' migration and differentiation, as well as enhance arteriogenesis and angiogenesis and reduce infarction volume, which contributed to the amelioration

of functional outcome after cerebral ischemia [18–20]. Our previous study also evidenced that simvastatin combined with MSCs could obviously activate astrocytes and increase astrocyte-derived stromal cell-derived factor-1α (SDF-1α), vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) expressions post-stroke, as well as up-regulate Akt/mammalian target of rapamycin (mTOR) signaling pathway in oxygen glucose deprived astrocytes [21]. Recently, the optimization of pharmacological agents in combination treatment began to transfer to Chinese medicines. Astragaloside IV, Naomai Yihao capsules, Buyang huanwu tang and Tongxinluo combined with MSCs had been demonstrated to notably promote angiogenesis and attenuate ischemic injury [22–25]. Based on angelica extract and active ingredients' multiple efficacy on ischemic stroke, our research group devotes the investigation of combination treatment of angelica and MSCs and finds some synergic functions and mechanisms.

2 Amelioration of Neurological Outcome and Reduction of Infarcted Volume

According to the evaluation of Garcia JH neurological score which included six sections: (1) evaluating animals' spontaneous activity; (2) symmetry of four limbs' movements when rat was held suspended by the tail and symmetric forepaws were assessed, (3) climbing wall of wire cage; (4) body proprioception; (5) reaction to touch on either side of the trunk; (6) response to vibrissa touch [26], we observed that derived from angelica's active ingredients, sodium ferulate (SF), as well as SF and n-Butylidenephthalide (BP) combined with MSCs began to improve neurological functional outcomes from day 3, and the increased trend always kept to day 7 after ischemia, suggesting the amelioration of neurological outcome was obviously superior to MSCs alone [27, 28]. Additionally, Bederson scale was administrated for neurological assessment following stroke, which included forelimb flexion, resistance to lateral push and circling behavior [29]. The scores are as below when SF (60 mg/kg) and BP (10 mg/kg) combined with MSCs (2×10^6 cells/ml, intravenous injection) were applied for the treatment of MCAo model in rats.

The scoring scale indicated that ischemic animals would have more significant neurological deficits than non-ischemic animals, resulting in a higher score. It indicated that neurological functional scores in SF + BP + MSC group began to reduce at day 3, showing neurological deficit had been attenuated (Table 12.1).

Magnetic resonance imaging (MRI) scanning and 2,3,5-triphenyltetrazolium chloride (TTC) staining showed whatever SF or SF and BP combined MSCs, both of therapeutic methods notably reduce infarcted volume post-stroke [30, 31].

Table 12.1 Bederson scale for neurological functional assessment (n = 20, means ± SD)

Group	3 h	1 day	3 days	7 days
Sham	0.02 ± 0.01	0.03 ± 0.03	0.02 ± 0.02	0.05 ± 0.02
MCAo	2.50 ± 0.21	2.56 ± 0.02	2.26 ± 0.14	2.08 ± 0.13
MSC	2.50 ± 0.12	2.64 ± 0.05	2.14 ± 0.12	1.45 ± 0.14*
SF + BP + MSC	2.50 ± 0.21	2.43 ± 0.17	1.35 ± 0.15*	1.09 ± 0.02*

*P < 0.05, vs. MCAo group

3 Acceleration of MSCs Differentiation and Migration

As a main organic acid in angelica, ferulic acid (FA) had been demonstrated that it could decrease infarction size and improve neurological function in MCAo rats through anti-oxidative and anti-inflammatory actions [32]. SF is the sodium salt of FA, which been used as an important agent for cardiovascular and cerebrovascular diseases. In 2005, Wang and colleagues firstly found that SF could induce Human MSCs to express neural proteins, such as nestin, neuron specific enolase and glial fibrillary acidic protein (GFAP), as well as advance MSCs to differentiate into neural-like cells in vitro [33]. Our previous study also indicated that SF could enhance 5-bromo-2′-deoxyuridine (BrdU)-labeled bone-derived MSCs to express nestin, GFAP and neuron-specific class III beta-tubulin (Tuj-1) in ischemic rat stroke model, suggesting it facilitated the differentiations of MSCs into astrocytic- and neuronal-like cells. Another experiment showed that adipose-derived MSCs were incubated with SF (5 μg/ml) and BP (0.75 μg/ml) for 3 and 7 days. Cultured MSCs in SF + BP group obviously presented neuronal morphology at day 3, and some cells even possessed long neuronal-like synapses under light microscopic observations. At day 7, fluorescence staining results also showed that combination of SF and BP could noticeable advance MSCs to express Tuj-1 (Fig. 12.1).

In order to illustrate the differentiated mechanisms, we investigated bone morphogenetic proteins (BMP) 2/4 and Notch-1 signaling pathways. As a member in the transforming growth factor beta superfamily, BMPs and their signaling systems play important roles in regulation of neural activity and rescue of injured neurons, and they could selectively and dose-dependently increase multipotent progenitor cells in murine embryonic subventricular zone (SVZ) to differentiate into astroglial lineage [34, 35]. It is reported that BMP2 and BMP7 levels began to enhance from 1 to 4 weeks in mice ischemic brain, which were associated with astrogliosis [36]. In our study, we found that SF combined with MSCs up-regulated BMP2/4 pathway post-stroke, which might be related to the differentiation of MSCs into astrocytic-like cells [30]. The mammalian family of Notch receptors consists of four members, Notch-1 through Notch-4, all of which are single pass transmembrane proteins. Study evidenced that activated Notch receptor promoted the survival and numbers of murine somatic and human embryonic stem cells by induced the expression of the specific target genes hairy and enhancer of split 3 (Hes3) and Sonic hedgehog, resulting in amelioration of motor skills after ischemic injury [37]. Not only Notch signaling plays a role in keeping the progenitors from differentiating into neurons,

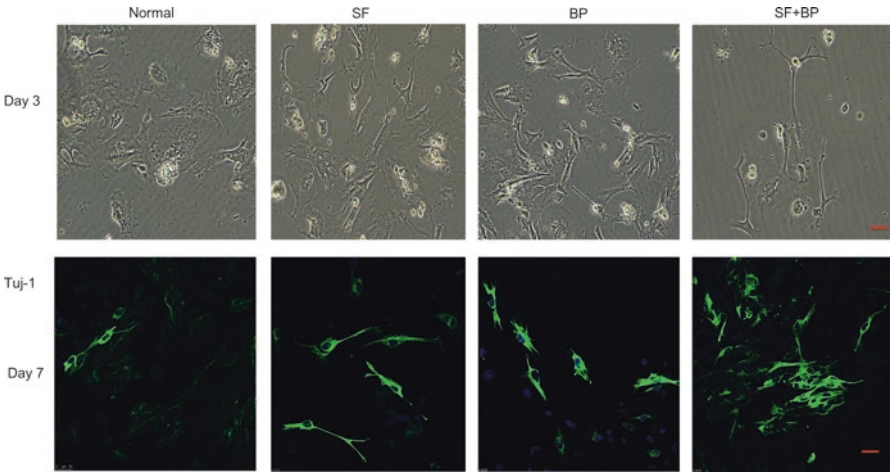

Fig. 12.1 Morphology and differentiation of cultured MSCs incubated with SF and BP. *First panel*: morphology of cultured adipose-derived MSCs incubated with SF and BP for 3 days under optical microscope; *Second panel*: immunofluorescence staining of Tuj-1 in MSCs after 7 days (scale bar: 20 μm)

but also down-regulated Notch1 signaling accelerated striatal astrocytes to carry a latent neurogenic program after stroke [38]. Moreover, there was cross-talk between Notch and BMP signaling pathways, which embodied that BMP2 enhanced Notch-induced transcriptional activation of Hes-5 and Hes-1 in mouse neuroepithelial cells [39]. In our study, it showed that the expressions of Notch-1, Hes1 and Hes5 in combination treatment of SF and MSCs group decreased, which might contribute to the differentiation of MSCs into neural-like cells [30].

Due to low migration efficiency of the transplanted BMSCs into the lesion area, MSCs treatment is limited. Wang and colleagues reported that SDF-1α and chemokine (CXC motif) receptor-4 (CXCR4) could systemically regulate transplanted MSCs towards ischemic zone in the MCAo rat model [40]. In the bone marrow, CXCR4 on endothelial cells and MSCs recruited peripheral blood SDF-1 to translocate into bone marrow, subsequently resulting in the homing of transplanted human $CD34^+$ hematopoietic progenitors to the bone marrow, and the effect was crucial related to SDF-1 gradient [41, 42]. Evidence indicated that up-regulated SDF-1/CXCR4 axis increased SVZ neuroblast cell migration after stroke [43]. Therefore, improving SDF-1α gradient in cerebral damaged tissue might contribute to MSCs recruitment into ischemic zone. Through Western blot and RT-PCR assay, it suggested that SF combined MSCs significantly up-regulate SDF-1/CXCR4 axis, which was beneficial to recruit more stem/progenitor cells to migrate into infarcted lesion [27]. Additionally, luciferase labeled adipose-derived MSCs was injected into the margin of laser illuminated area in photochemically induced stroke model, and it showed that SF and BP could obviously promote MSCs' abilities of survival and migration (Fig. 12.2).

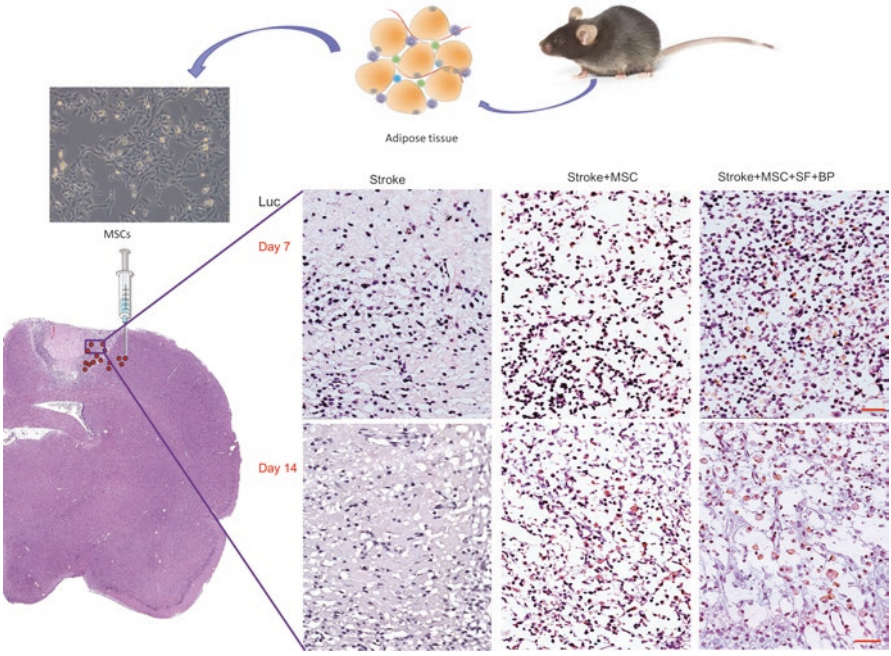

Fig. 12.2 Migration of Luciferase labeled adipose-derived MSCs at day 7 and 14 post-stroke. Luciferase immunohistochemistry staining images suggested that SF and BP could advance migration of adipose-derived MSCs into infarcted zone (scale bar: 50 μm)

4 Enhancement of Angiogenesis and Neurogenesis

Being a potential therapeutic candidate in the treatment of ischemic stroke, MSCs are capable of promoting angiogenesis and neurogenesis after cerebral ischemia [11, 44, 45]. Exogenous transplanted MSCs not only directly differentiated vascular endothelial cells (ECs), but also induced endogenous angiogenic responses to amplify angiogenesis and vascular stabilization after stroke [11, 46]. In addition, angiogenetic gene-modified MSCs, e.g. MSCs transfected with the angiopoietin-1, placental growth factor, VEGF and Flk-1 gene showed the greatest structural-functional recovery and notably angiogenesis and neurogenesis post-stroke [47–49]. Komatsu and colleagues thought that angiogenesis accounted for the main therapeutic effects, although there were several hypotheses in the treatment of MSCs [50]. Our previous review summarized the actions of new vessel formation after ischemic stroke, which included the improvement of cerebral blood flow (CBF) and metabolism in infarction lesion, removal of necrotic debris, enhancement of neurotrophic components for neuronal remodeling and endogenous stem/progenitor cells migration [51]. Based on the efficacy of angiogenesis, it has been recognized to be the basis and prerequisite for neurogenesis. However, not all angiogenesis is advantageous for stroke by MSCs transplantation. In Type 1 diabetic MCAo rats, angiogenesis by grafted MSCs deteriorated internal carotid artery neointimal formation and blood-brain barrier (BBB)

leakage, which possibly was due to increased expression of angiogenin. The adverse effects promoted mortality and the risk of brain hemorrhage [52]. Thereby, the homeostasis of angiogenesis should be taken into account after cerebral ischemic stroke.

More and more evidences indicated that the occurrences of angiogenesis and neurogenesis were coupled processes rather than separate after stroke [53, 54]. It suggested that coculture of neural progenitor cells from SVZ with cerebral ECs from the stroke boundary notably increased neural progenitor cell proliferation and neuronal differentiation, and inhibition of VEGF receptor 2 decreased these beneficial effects on neurogenesis and angiogenesis [53]. Sun and colleagues also demonstrated that VEGF exerted primary role in neuroprotection, survival of new neurons and angiogenesis after cerebral ischemia [55]. In addition to promoting synaptic and axonal plasticity and advancing neurogenesis of MSCs, BDNF also involved in angiogenesis after stroke [56–58]. Phosphorylated AKT could activate mTOR in sequent up-regulate Hypoxia-inducible factors (HIF)-1α expression, consequently improve VEGF expression which contributed to angiogenesis post-stroke, another hand AKT/mTOR had been demonstrated as a crucial target to regulate new neuron development and was essential to maintain endogenous neuronal progenitor pool [59, 60]. Additionally, reports also showed that BDNF could bind to Tropomyosin receptor kinase B (TrkB) receptor and then activate AKT/mTOR signaling resulted in neuroprotective actions in stroke [61, 62]. AKT/mTOR pathway is a central regulated approach of angiogenesis.

Evidence showed that angelica extract and active ingredients were able to improve angiogenesis. Lam and colleagues demonstrated that human umbilical vein endothelial cells (HUVECs) and zebra fish intestine capillaries incubated with angelica extract presented obvious angiogenic abilities, whose mechanism was mainly related to p38 and Jun N-terminal protein kinase 1/2 phosphorylation [63]. FA could advance HUVECs to secret VEGF, platelet-derived growth factor and HIF-1α, consequently, promote the ability of angiogenesis via activation of PI3K signaling pathway [64]. As a new drug for cerebralvascular disease approved by China FDA, NBP was capable of increasing brain microvessels density against stroke, whose mechanisms were associated with enhanced expressions of VEGF, VEGFR and HIF-1α as well as activation of extracellular signal-regulated kinases (ERK)1/2 and PI3K/Akt-endothelial nitric oxide synthase (eNOS) signal pathways [65–68].

It has been observed that angiogenesis and neurogenesis simultaneously taken place in the penumbra, and newly born, immature neurons derived from neural stem cells (NSCs) in SVZ and dentate gyrus (DG) closely associate with the remodeling vasculature in this neurovascular niche [69]. In rat permanent bilateral common carotid artery occlusion model, angelica extract that contained the component Z-ligustilide improved neurogenesis in the hippocampus and cognitive decline due to hypoperfusion though enhanced expressions of BDNF and phosphorylated cyclic adenosine monophosphate-responsive element binding protein and γ-aminobutyric acid [70]. In vitro, FA promoted proliferated ability of cultured neural stem/progenitor cells derived from embryonic telencephalon and the number and size of secondary formed neurospheres; in vivo, it increased the number of newly generated cells in the hippocampal DG of corticosterone-treated mice [71]. Previous study suggest that BP, a kind of alkylphthalide derived from the volatile oil of angelica, had ECs protective, vasorelaxing, antiplatelet and antianginal effects, as well as maintained stem

cells pluripotency [72–76]. Based on the characteristics of SF and BP, we chose SF and BP as representative constituents of angelica and discuss the effects and mechanisms combined with BMSC on angiogenesis and neurogenesis after ischemic stroke. In our study, we think that SF and BP was a "Trigger point" which embodied that they advanced MSCs to synthesize VEGF and BDNF, subsequently AKT/mTOR cascade was activated in cerebral parenchymal cells, consequently the combination treatment improved angiogenesis and neurogenesis. In order to define the role of astrocytes on angiogenesis in the treatment of MSCs combined with SF and BP, we investigated astrocyte-derived neurovascular trophic factors and found that combined treatment could obviously increase the expressions of astrocyte-derived VEGF and BDNF via activation of astrocytic AKT/mTOR signaling, resulting in migration and tube formation of HUVECs [28, 31]. Additionally, Immunohistochemistry staining images indicated that adipose-derived MSCs combined SF and BP promoted Von Willebrand factor $(vWF)^+$ capillary density compared with SF + BP group and MSC group and Western blotting showed combination treatment notably enhance Doublecortin (DCX) expression in ischemic boundary zone, suggesting the enhancements of angiogenesis and neurogenesis (Fig. 12.3).

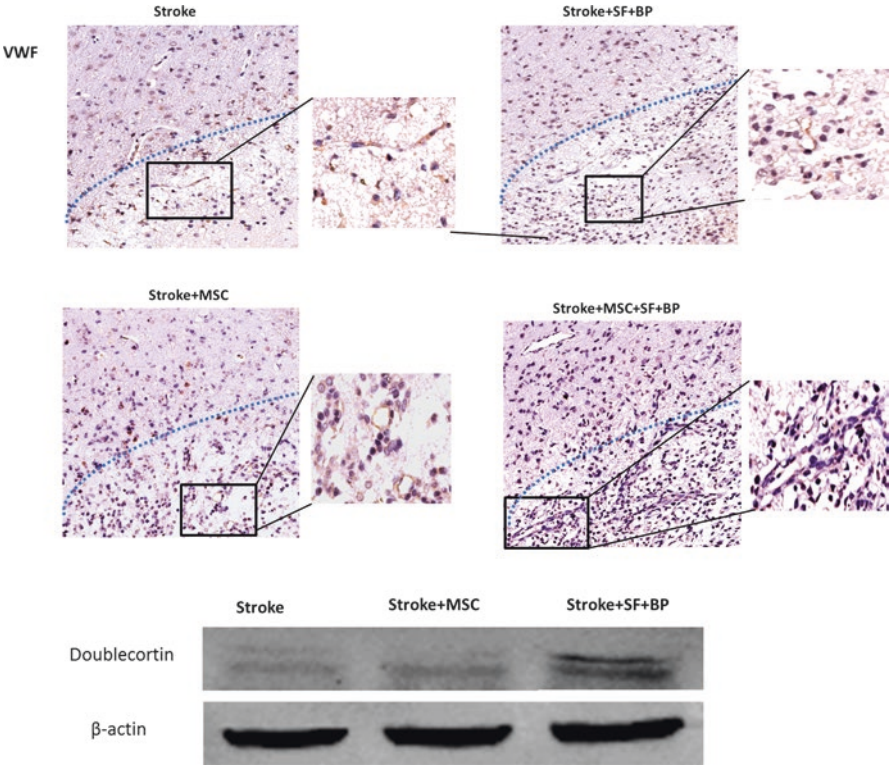

Fig. 12.3 vWF positive capillary and DCX expression in ischemic boundary zone after ischemia. Immunohistochemistry staining images and western blotting showed SF and BP combined with MSCs significantly improve angiogenesis and neurogenesis

5 Regulation of Astrocytes, Activation or Inhibition?

Evidences indicate reactive astrocytes play an important neuroprotective role through enhanced number of mitochondria and antioxidant enzyme activity, reabsorption of glutamate, antitoxic action of free radicals and anti-apoptosis, and regulating immunological response against ischemic brain injury [77]. In the study of cerebral energy metabolism, Kajihara and colleagues demonstrated that astrocytes increased cytoplasmic storage capacity of glycogen granules in the ischemic penumbra after ischemic stroke, and protoplasmic astrocytes gradually became into fibrous astrocytes as ischemic time went by [78]. Previous evidence showed that MSCs treatment could reduce thickness of glial scar formation by reactive astrocytes, consequently decrease inhibition of axonal and synaptic growth, as well as neuronal functional regeneration in the later stage of cerebral ischemia [79, 80]. However, the newest report suggested that scar formation by regulation of astrocyte had advantageous action for axonal regeneration in severe spinal cord injury [81]. On the other hand, in the maintenance of BBB integrity, reactive astrocytes are traditionally thought as detrimental actions which present promoted endothelial permeability and VEGF secretion, as well as decreased occludin and claudin-5 proteins expressions [82–85]. Therefore, it should be compromised evaluation between the beneficial and adverse effects of reactive astrocytes post-stroke.

Present study showed that NBP reduced GFAP-positive astrocytes induced by chronic cerebral ischemia, and inhibited the amyloid β (Aβ)-induced astrocyte activation and pro-inflammatory molecules, which contributed to against ischemic stroke and Alzheimer's disease [16, 86]. Our previous study indicated that SF and bone-derived MSCs respectively activate astrocytes at day 3, and SF combined with MSCs more significantly promoted GFAP expression in ischemic penumbra. Interestingly, when SF and BP combined with adipose-derived MSCs were used to treat photothrombotic stroke, we found SF and BP notably inhibited activation of astrocytes in ischemic boundary zone, but adipose-derived MSCs activated astrocytes at day 7 after ischemia. Simultaneously, the combination treatment also suppressed GFAP expression to a certain extent (Fig. 12.4). The results suggested that different composition of angelica might exert differential actions on astrocytes post-stroke.

The detrimental effect of reactive astrocytes on BBB permeability mainly attributed to its VEGF secretion. Study indicated that knockdown of VEGF no longer damaged endothelial barrier, so astrocyte-derived VEGF has been described as a key mechanism in BBB breakdown [84]. In addition to reactive astrocytes, adult human dental pulp stem cells also were found to secret VEGF-A resulted in enhancement of permeability across an in vitro model of BBB [87]. Shimotake and colleagues demonstrated that VEGF receptor-2 inhibition advanced ischemic injury and reduced endothelial cell proliferation in neonatal rats, whereas it attenuated BBB permeability in diabetic mice after stroke, whose action was associated with enhanced endothelial transcytosis rather than tight junctions [88, 89]. Recent report evidenced that astrocyte-derived Pentraxin 3 bound to VEGF, subsequently notably decreased VEGF-induced endothelial permeability in vitro, and astrocytes existed at least two subclasses by different migratory abilities after

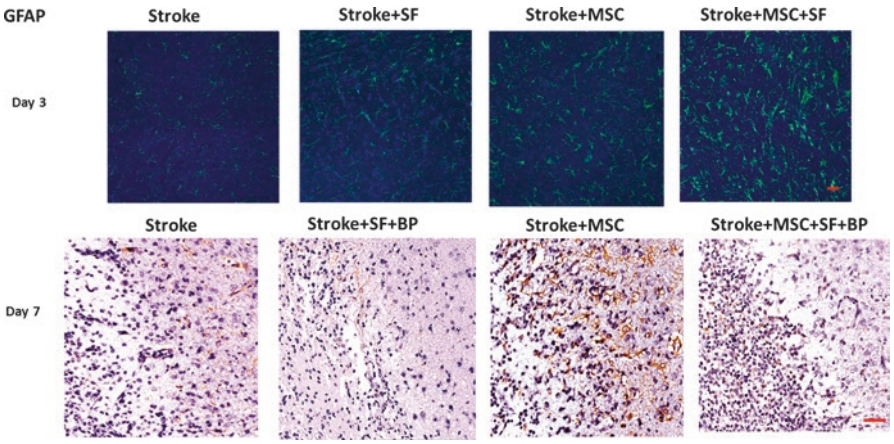

Fig. 12.4 Angelica' active ingredients combined with MSCs regulated GFAP expression in penumbra post-stroke. *First panel*: Immunofluorescence staining indicated that combining SF and bone-derived MSCs could activate astrocyte after 3 days in MCAo model; *Second panel*: SF and BP combined with adipose-derived MSCs inhibited reactive astrocytes after 7 days in photothrombotic stroke model (scale bar: 50 μm)

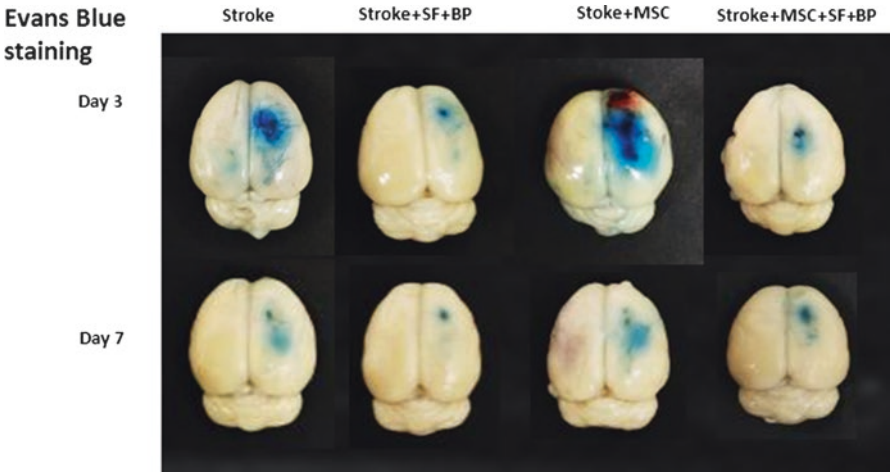

Fig. 12.5 Evaluation of blood-brain-barrier integrity after photothrombotic stroke. Representative images of brain EB staining in rat at day 3 and 7 were presented

cerebral stoke [90, 91]. Therefore, the results reflect the multifaceted actions of VEGF and astrocytes on BBB integrity and endothelial function. In our study, Evans blue staining showed that SF + BP group distinctly reduced BBB leakage compared with other three groups whatever at day 3 or 7 after stroke, which might attribute to inhibitions of astrocytes activation, and SF and BP combined with MSCs presented the effect of maintenance of BBB integrity which was notably superior to MSC alone treatment, suggesting that SF and BP could be against side effect of increased BBB leakage of adipose-derived MSCs (Fig. 12.5). But VEGF

expression in combination treatment was the highest (Data no show), whether the therapy influenced subtypes of astrocytes, astrocyte-derived Pentraxin 3, endothelial function or regulation of VEGF receptors, exact mechanisms need to be further illustrated in future experiment.

6 Improvement of Cerebral Blood Flow and Glucose Metabolism

Neurovascular coupling is responsible for controlling regional CBF by neurons directly or astrocytes indirectly secreting vasodilator factors targeting on vascular cells under physiological condition [92]. Moreover, it reported that reactive astrocytes exerted key effect on rCBF regulation, and they could modulate rCBF longer than neurons under pathological conditions [93]. We examined perfusion-weighted imaging (PWI) by MRI to evaluate CBF after photothrombotic stroke. It showed that SF and BP combined with adipose-derived MSCs could significantly ameliorate CBF in the infarction zone (Fig. 12.6a). Previous study had demonstrated that the combination treatment could significantly enhance angiogenesis and neurogenesis, as well as regulate astrocytes, so we thought the interactions of newly neuron, astrocyte and neovascularization contributed to enhancement of CBF in infarction. Additionally, ^{18}F-2-deoxy-glucose (FDG)-positron emission tomography–computed tomography (PET/CT) was administrated to assess glucose metabolism. As shown in Fig. 12.6b, the cortical metabolic defect partially recovered with the time prolongation, and it seemed to more obvious amelioration in MSC + SF + BP groups at day 14, suggesting that combination treatment could enhance glucose metabolism. We also observed that combing SF and MSCs promoted Glucose transporter 1 expression in ischemic boundary zone [30], which might be a mechanism of glucose metabolism.

In summary, these studies uncovered that angelica' active ingredients could advance MSCs migration and differentiation, and combining MSCs with angelica' active ingredients ameliorated neurological function and reduced infarction volume, improved neovascularization and neurogenesis, regulate astrocyte characteristics, enhanced CBF and glucose metabolism, and decrease BBB permeability, which restitute the structure and function of neurovascular unit in infarction zone after stroke. Therefore, the combination treatment should be a more effective therapy due to synergic functions of supplementary approaches.

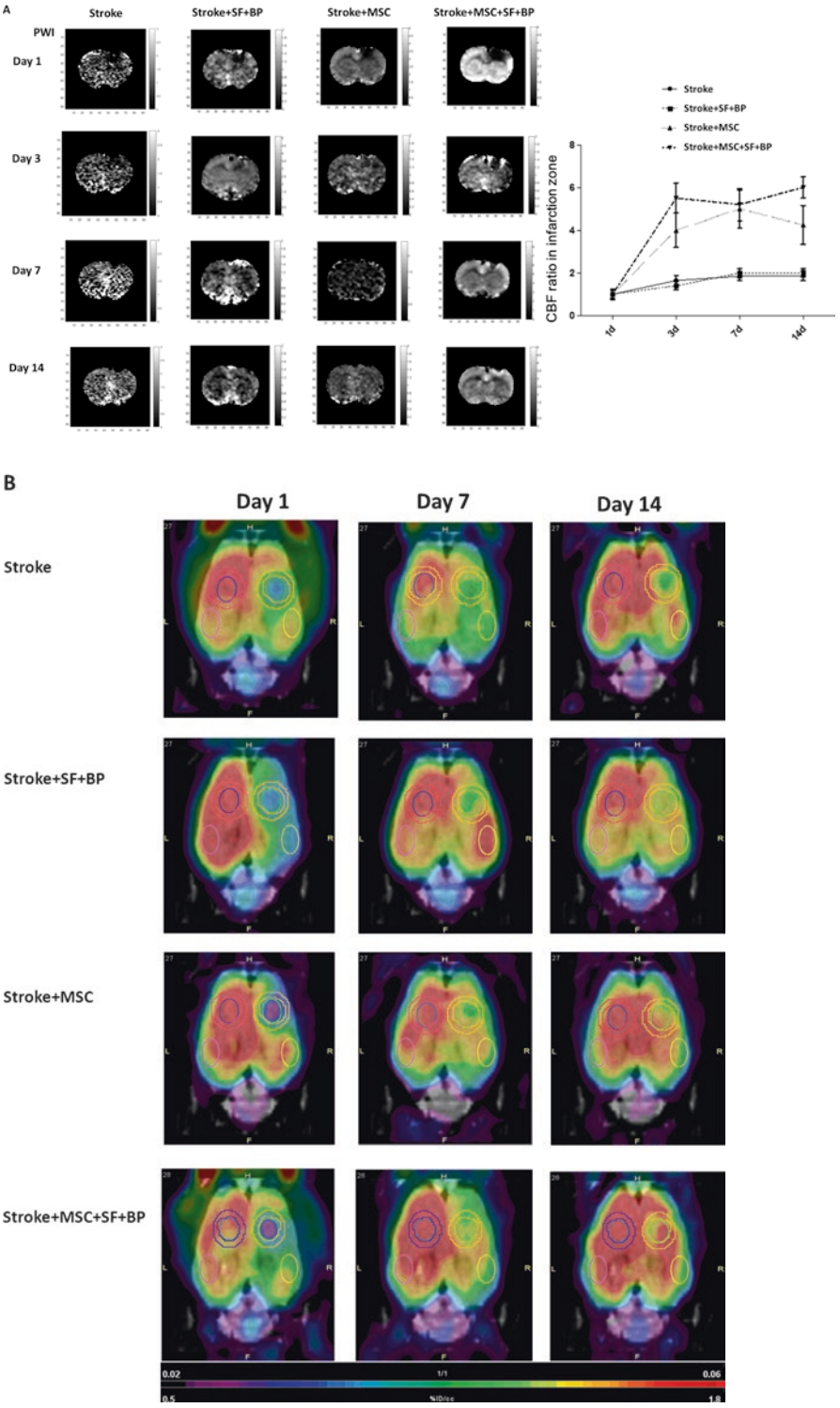

Fig. 12.6 Evolution of cerebral blood flow and glucose metabolism in infarct lesion. (**a**) Images of PWI at day 1, 3, 7 and 14 after ischemia were presented and quantitative analysis of CBF. (**b**) ^{18}F-FDG PET imaging at day 1, 7 and 14 was showed

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