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E-mail: cjim_en@cjim.cn

Feature Article

Essential Role of Chinese Medicines in Mesenchymal Stem Cells Transplantation for Treatment of Ischemic Stroke

ZHAO Yong-hua

ABTRACT Ischemic stroke accounts for the majority of all strokes and has been primary causes of long-term disability and mortality in worldwide. Mesenchymal stem cell (MSC) therapy suggests significantly improved effects on neurological functional outcome, neurogenesis, angiogenesis, blood-brain barrier permeability, inflammatory injury, neuroprotection and so on, following stroke. However, the occurrence of adverse effects results in restriction of the therapy. Chinese medicine accumulates abundant clinical experiences on stroke for over two thousand years, and some formulae and active ingredients of Chinese medicines have presented obvious efficacies in clinical treatment. Therefore, based on Chinese medicine theory, we provide some ideas of screening agents for combination treatment of Chinese medicines and MSC for ischemic stroke, and summarize the potentials of Chinese medicines in MSC treatment and analyze the feasibilities of Chinese medicines against side effects of MSC therapy. Consequently, we propose Chinese medicines combing with MSC should be a promising approach to clinical stroke treatment in future.

KEYWORDS Chinese medicines, combination treatment, ischemic stroke, mesenchymal stem cell

The data of national epidemiological survey of stroke in China indicate that the prevalence and incidence of stroke are significantly higher in 2012 to 2013 than in 1985, and ischemic stroke (incidence rate: 166.9/100,000) accounts for main pathological types of stroke.[1] According to 2018 Guidelines for the Early Management of Patients With Acute Ischemic Stroke, standard therapeutic methods of arterial recanalization in acute ischemic stroke are intravenous Alteplase and/or mechanical thrombectomy. However, even if in United States, only 6.5% patients with ischemic stroke can receive thrombolytic therapy of tissue plasminogen activator due to missing therapeutic window, individual contraindications and failing to pay higher costs in hospitals.[2] In addition, the guidelines do not recommend neuroprotective agents for patients within acute stage, because they suggest negative results on amelioration of neurological function during clinical trials. Therefore, it is very necessary to develop novel promising therapeutic approach to treatment of ischemic stroke.

Mesenchymal stem cells (MSCs), as the most common candidate of Cell-based therapies, have showed to attenuate neurological functional injury after cerebral ischemia via direct lesion tissue replacement and secretion of paracrine factors, so called "bystander effects".[3] Some ischemic stroke clinical tests using MSC treatment in phase Ⅰ and phase Ⅱ have already been performed in USA, China, France, Europe and so on, and it suggests that intravenous injection of MSC improves patients' function evaluated by the Barthel Index (BI), the National Institutes of Health Stroke Score (NIHSS) and the modified Rankin Scale.[4] Moreover, in India, 6 patients with chronic stroke by intravenous transplantation of autologous MSCs show a statistical significance in their activities of daily living measured with modified BI after 4 years compared with control group, but there are no obvious improvements on muscle power and tone recovery.[5] Although the results of clinical trials and meta-analysis suggest it appears to safety during the process of MSC therapy, there are some literatures to report adverse events of fever, seizures, pain, immunosuppression, hemorrhage, microembolism and

State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao (999078), China
Tel: 853-88224877, Fax: 853-28841358, E-mail: yonghuazhao@um.edu.mo
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so on.[6,7] It is necessary to further define the safety profile of MSCs with rigorously evaluated scales in clinical trials of ischemic stroke.

Due to complicated pathological involution of ischemic stroke, a combination treatment of stem cells and drugs rather than a stand-alone therapy is more appropriate.[8] Chinese medicine has a long-term history of over 2,500 years, which accumulates numerous medicinal prescriptions and pharmacological agents and abundant clinical therapeutic experiences on ischemic stroke. So combing MSCs with Chinese medicines should be more superior to either therapy alone.

Precision Screening of Chinese Medicines based on Chinese Medicine Theory

Based on the therapeutic principle of dispelling stasis to promote regeneration, blood- activating and stasis-resolving medicinals and qi and blood-tonifying medicinals might be candidate drugs. Danshen Injection (丹参注射液), salvia mtiorrhiza, sodium ferulate (SF) and Astragalus, ginsenoside Rgl are be demonstrated to induce MSCs differentiation into neuron-like cells.[9] Tongxinluo (通心络) can promote MSCs' angiogenic ability by up-regulation of matrix metalloproteinase-2 and vascular endothelial growth factor (VEGF) expressions.[10]

Based on the therapeutic principle of nourishing Shen (Kidney) and filling essence, tonifying Shen medicinals are available for combination treatment. As a main active ingredient of *Radix Rehmanniae* root, Catalpol has been potential neuroprotective agent for stroke treatment via anti-oxidation, anti-inflammation, anti-apoptosis and suppressing autophagy properties.[11] Moreover, a new research finds that catalpol can enhance VEGF secretion and survival of oxygen glucose deprived MSCs.[12]

Based on the pathological characteristics of mental confusion and unconsciousness, resuscitative medicinals might be one option for combination treatment. Borneol, an organic compound found in Dipterocarpaceae plants, combing with MSCs notably decrease infarction volume and apoptosis, increase neurogenesis and ameliorate the functional outcome compared with MSCs alone.[13] Moschus combined with borneolum synthcticum presents significant anti-apoptotic activity and neuroprotective effects on ischemia stroke.[14]

Based on the pathological characteristic of cerebral collaterals injured by toxin (毒损脑络), heat-clearing and detoxicating medicinals (清热解毒药) might be selected for the combination treatment. Sanhuang Xiexin Decoction (三黄泻心汤) is composed of *Coptidis Rhizoma*, *Rhei Radix* et *Rhizoma*, *Lophatherum Gracile*, *Bile Arisaema* and *Forsythia*, which decreases the syndrome of fire-heat scores and inflammatory factors in patients with acute ischemic stroke.[15] As the main active composition of *Coptidis Rhizoma*, berberine can be against oxidative stress-induced apoptosis of MSCs.[16]

Chinese Medicines Contribute to MSCs' Benefit Effects Exertion by Regulation of Cerebral Microenvironment Post-stroke

Rehabilitating Blood-brain Barrier Integrity

Increasing blood-brain barrier (BBB) permeability not only causes brain edema exacerbation, but also aggravates inflammatory injury and increases hemorrhagic risk following stroke. Evidence indicates that the water extract of *Angelica gigas* hairy roots (AG) attenuates BBB permeability by enhancing the expressions of ZO-1 and occludin after ischemic stroke, so combination treatment of AG and MSCs exerts more obvious protective role in BBB integrity than MSCs alone.[17,18]

Anti-inflammation

Stroke-induced microglial cells are activated and subsequently secrete pro-inflammatory factors involved in BBB leakage and neuronal injury. Wogonin derived from *Scutellaria baicalensis* Georgi suppresses microglial activation by down-regulating nuclear factor kappa B (NF-kappaB) signal pathway,[19] and Astragalus and Baicalein attenuate the levels of cytokine IL-1β, IL-8, and tumor necrosis factor-α (TNF-α) in lipopolysaccharide (LPS)-induced MSCs,[20] suggesting that Chinese medicinal active compounds have significant anti-inflammatory efficacy in stroke treatment.

Pro-angiogenesis

Therapeutic angiogenesis exerts multiple actions on ischemic stroke, e.g. transportation of tropic substances, migration of progenitor/stem cells and removal of necrotic debris. Buyang Huanwu Decoction (BHD, 补阳还五汤), a classical tonifying qi and resolving stasis formula, increases angiogenesis by up-regulating silent information regulator 1/VEGF pathway

after cerebral ischemia/reperfusion.[21] Combination treatment of BHD and MSCs further boosts exosomes derived from MSCs to enhance angiogenesis after focal cerebral ischemia.[22]

Chinese Medicines Facilitate Migration and Proliferation of MSCs

MSCs homing to infarcted zone contributes to the exertions of direct and indirect therapeutic functions post-stroke. Tanshinone ⅡA- and astragaloside Ⅳ-stimulated MSCs highly express CXCR4 and the two agents facilitate MSCs migration toward a stromal cell-derived factor-1α gradient in lower chamber of transwell system.[23] Additionally, our study demonstrates that SF, n-butylidenephthalide (BP), two active ingredients of *Radix Angelica* sinensis, can advance the proliferative ability of adipose-derived MSCs by intracerebral injection into peripheral ischemic zone.[24]

Chinese Medicines Advance Differentiation of MSCs

As pluripotent cells, MSCs display the ability to differentiate into multiple cellular types. Studies suggest that baicalin and SF respectively induce the differentiation of MSCs into neural-like cells *in vitro*.[25,26] *In vivo*, our previous study found SF could not only advance Brdu-labeled MSCs differentiation into neural-like cells, but also elevate their astrocytic-like cellular differentiated ability after cerebral ischemia.[27]

Chinese Medicines Boost Autocrine and Paracrine Functions of MSCs

Due to the limited number of MSCs in the brain by intravenous delivery, it raises doubt on the importance of the replacement of injured neurons with direct differentiation of MSCs. Present consensus is that the factors secreted by MSCs and its bystander effects play important roles in neurological functional recovery. In our studies evidence SF and BP are a "Trigger point", which embodies that they facilitate MSCs to synthesize VEGF and brain-derived neurotrophic factor, subsequently activate astrocytic AKT/mTOR signal and consequently promote angiogenesis post-stroke.[28,29]

Chinese Medicines Conduce to Attenuating Side Effects of MSCs

Ameliorating MSC-Induced Immunosuppression

Immunosuppression increases stroke patients' susceptibility to infections, which also is one important cause of aggravation/death post-stroke. Although transplantation of MSC did not exacerbate the natural course of stroke-induced immunodepression in C57BL/6 mice,[30] there is no evidence to demonstrate the immune safety of MSC administration in clinical trials at present. Ginsenoside-Rd, as a specifically anti-inflammatory and immune-modulating drug, has been applied in phase Ⅱ or phase Ⅲ clinical trials for acute stroke, and suggests distinctly improve NIHSS and modified Rankin Scale in patients.[31] So researchers might take advantage of ginsenoside-Rd against MSCs' immunosuppressive results.

Reducing the Risk of Hemorrhagic Transformation

Chen and colleagues found that instead of amelioration of neurological outcome, grafted MSCs increased BBB permeability resulted in cerebral hemorrhage (CH) and high mortality in type 1 diabetic rats.[32] A clinical trial result indicates panax notoginseng saponins (PNS) significantly improve NIHSS and BI in patients with CH after 3 weeks treatment, and the drug can mobilize bone-derived MSCs to migrate into peripheral blood in cerebral ischemic rats.[33,34] In addition, PNS also shows anti-diabetic potential, so it should be a suitable candidate combined with MSC for ischemic stroke treatment in patients with diabetes mellitus.

Prevention of Microembolism by Improvement of Cerebral Blood Flow

Because intravenously delivered MSCs mainly accumulates in lung and liver, intra-arterial cell infusion becomes an efficient delivery route of targeting to ischemic cerebral tissues. However, evidence showed that the number of embolisms increased and cerebral blood flow (CBF) reduced with MSC dose by intra-arterial graft, which notably exacerbated lesion size in middle cerebral artery occlusion rats.[35] Currently, study indicates that capillary pericytes exerts essential actions on regulation of dilate and constrict capillaries resulted in the change of CBF and some Chinese medicines have suggested protective effects on pericytes in microvascular dysfunction.[36,37] Together with anti-platelet Chinese medicinal agents, it will contribute to the attenuation of microembolism in MSC treatment for stroke.

In summary, MSC transplantation is a promising approach to patients with cerebral ischemia, but

some limitations of MSC application for treatment are still comprehensively understood in order to ensure clinical safety and efficacy. Therefore, developing standardized technologies and guidelines from isolation to infusion is a prerequisite to quality assurance for clinical-grade MSCs.[38] Based on Chinese medicines multiple beneficial features on MSC therapy, combination treatment of MSC and Chinese medicines for ischemic stroke will have a broad clinical applied outlook in future.

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