Potential advantages of a combination of Chinese Medicine and bone marrow mesenchymal stem cell transplantation for removing blood stasis and stimulating neogenesis during ischemic stroke treatment

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Abstract

Combined treatment of ischemic stroke with Chinese medicine and exogenous bone marrow mesenchymal stem cell (BMSC) transplantation may improve the removal of blood stasis and stimulation of neogenesis. Chinese medicines that remove blood stasis not only promote blood circulation but also calm the endopathic wind, remove heat, resolve phlegm, remove toxic substances and strengthen body resistance. The medicinal targeting effect of Chinese medicine can promote the homing of BMSCs, and the synergistic therapeutic effects of drugs can contribute to BMSC differentiation. As such, exogenous BMSC transplantation has potential advantages for neogenesis. Chinese medicines and exogenous BMSCs provide complementary functions for the removal of blood stasis and stimulation of neogenesis. Therefore, a combination of Chinese medicine and transplantation of exogenous BMSCs may be particularly suited to ischemic stroke treatment.

INTRODUCTION

The therapeutic methods for removing blood stasis and stimulating neogenesis were developed on the basis that new blood is not generated in the presence of blood stasis. The stimulation of neogenesis is performed simultaneously with the removal of blood stasis to cure diseases and restore body energy[1]. Fu Qing-Zhu, a great authority on gynecology who lived between the end of the Ming Dynasty and the first years of the Qing Dynasty, effectively applied this method, and the Shenghua Decoction he developed was used as a representative recipe for removing blood stasis and stimulating neogenesis in later ages. In recent years, experimental studies on ischemic stroke have demonstrated that Chinese medicines used for removing blood stasis and stimulating neogenesis can increase the density of CD31 positive microvessels following middle cerebral artery occlusion (MCAO) in rat brain, and also promote angiogenesis to a certain degree[2]. However, clinical data indicate that the use of Chinese medicines alone do not provide satisfactory results in ischemic stroke treatment.

Since the late 1990s, stem cells have become increasing...
ly important to medical studies. This is because of their ability to migrate, proliferate and differentiate following transplantation. Human bone marrow-derived mesenchymal stem cells (hBMSCs) are suitable for autologous transplantation in clinical practice because they are easily obtained from patients and do not induce an immune reaction. Many studies have demonstrated that transplantation of BMSCs is able to repair nerve cells injured by cerebral ischemia and subsequently improve neurological function. Therefore, BMSC transplantation is considered an appropriate new treatment for ischemic cerebrovascular diseases. In recent years, Chinese medicine has played an increasingly important role in treatment involving BMSC transplantation. For example, the TCM concept of wholism has influenced the culture, differentiation and migration of stem cells. The concept of preventing the onset and deterioration of disease has influenced the migration of BMSCs. The Chinese medicine characteristics of multi-causes and steady effects, multi-targets, low toxicity or minimal side effects, may play an important role in stem cell transplantation. Therefore, exploring the use of Chinese medicines combined with exogenous BMSC transplantation for the treatment of ischemic cerebrovascular diseases is of great importance, because the method for removing blood stasis and stimulating neogenesis is considered to conform to the TCM theory for the pathogenesis of ischemic stroke. Ischemic stroke treatment with Chinese medicines combined with exogenous BMSC transplantation may enable improved removal of blood stasis and stimulation of neogenesis. Potential Advantages of Removing Blood Stasis Following Ischemic Stroke by Combined Treatment with Chinese Medicine and Exogenous BMSC Transplantation According to TCM, the pathogenesis of ischemic stroke follows a dynamic development course, which goes through various stages including Zang- and Fu-organ functional abnormalities, Qi blood and body fluid disorders, and formation of poisons that affect the brain and injure collaterals. The pathological products and pathogenic factors of wind, fire, phlegm, blood stasis and poison, arise one after another and continually injure the brain collaterals, brain marrow and mentality to induce confusion and unconsciousness. Therefore, the removal of wind, fire, phlegm, blood stasis and poison following their onset may alleviate injury to mental activity and Zang- and Fu-organs. Removal of blood stasis should not be considered a way of promoting blood circulation only, but also for calming the endopathic wind, removing heat, resolving phlegm, removing toxic substances and strengthening body resistance. For the treatment of ischemic cerebrovascular diseases with Chinese medicines combined with exogenous BMSC transplantation, the selection of the Chinese medicine should not be limited only to drugs that remove blood stasis by promoting blood circulation. Various options are discussed below.

**Chinese medicines for supplementing or invigorating Qi and promoting blood circulation**

As the active component of classical Buzhong Yiqi Decoction, hexadecanoic acid may promote the proliferation and migration of BMSCs. For example, in MCAO rats treated with Buyang Huanwu Decoction in combination with BMSC transplantation, the infarction zone was significantly reduced and neurological functional recovery was promoted.

**Chinese medicines for supplementing Qi, promoting blood circulation and resolving phlegm**

Naomai Capsule I is a Chinese medicine compound that functions to supplement Qi, activate blood circulation and resolve phlegm. In a study on the effects of Naomai Capsule I combined with BMSC transplantation on angiogenesis and neurological function in the rat following cerebral ischemia and reperfusion, it was found that Naomai Capsule I could significantly increase CD31 expression in the ischemic lesion zone. These results indicated that this drug could induce and promote angiogenesis, and improve neurological function, following BMSC transplantation. Moreover, the effects became more obvious as the therapeutic time course increased.

**Chinese medicines for removing heat and toxic substances**

The effects of rhubarb aglycone, which is obtained by ethanol extraction of Dahuang (Radix et Rhizoma Rhei), combined with BMSC transplantation was tested in the rat model of MCAO. This treatment regulated the matrix metalloproteinase-9 (MMP-9) imbalance by increasing the expression of tissue inhibitor of metalloproteinase-1 (TIMP-1), which reduced the degradation of collagen IV (Col IV) in microvascular basement membrane at different stages following BMSC transplantation. Subsequently, permeabilization of the blood–brain barrier (BBB) was alleviated, enabling it to perform its protective function.

**Chinese medicines for supplementing Qi, promoting blood circulation and removing toxic substances**

Naomai Tong functions to supplement Qi, promote blood circulation and remove toxic substances. L1 Jian-sheng, et al. reported that Naomai Tong combined with BMSC transplantation could improve cerebral circulation in rat brain and protect tissue from injury. These effects possibly involved the down-regulation of Fas and FasL expression during the early stages of cerebral ischemia, followed by inhibition of caspase-3-induced apoptosis of nerve cells.

**Chinese medicines for tonifying the kidney**

Administration of BMSCs incubated with Dihuang Yinzi promoted HSP70 and vascular endothelial growth factor (VEGF) expression in the rat following...
cerebral infarction. It was also found that Yougui Pill, Zuogui Pill, Dihuang Yinzi, retinoic acid (RA) and recipes for tonifying the kidney combined with RA could promote the secretion of nerve growth factor-beta (NGF-β), brain-derived neurotrophic factor (BDNF) and VEGF by BMSCs. The classical prescriptions for tonifying the kidney-yin and both the kidney-yin and yang were superior to the classical prescription for tonifying kidney-yang in promoting BDNF or VEGF secretion by BMSCs.

Potential advantages of exogenous BMSC transplantation for neogenesis

Studies have shown that following cerebral infarction in rats, some Chinese medicines such as Zhutan Tongluo Prescription promote the proliferation of endogenous stem cells, which then differentiate into neurons that promote neurological function recovery. However, the biological activities of endogenous neural stem cells are influenced by ischemic injury, and the repair of nervous injury and improvement of neurological function are therefore limited. The traditional method for removing blood stasis and stimulating neogenesis does not achieve satisfactory results in the repair of nerve injury induced by local cerebral ischemia, and so exogenous BMSC transplantation is potentially advantageous. In addition to removing blood stasis, Chinese medicine has a medicinal targeting effect that can promote the successful migration and differentiation of exogenous BMSCs to further stimulate neogenesis.

The Medicinal targeting effect of Chinese medicine can promote the homing of BMSCs

Many studies have shown that BMSCs are able to migrate into ischemic tissue. Following local cerebral ischemia, destruction of the BBB may possibly contribute to the selective migration of BMSCs into the ischemic zone. In addition, inflammatory injury of ischemic brain tissue and the expression of many chemotactic cytokines, particularly stromal cell-derived factor-1 (SDF-1) and its specific receptor, chemokine (CXC motif) receptor-4 (CXCR4), play an important role during the interaction of the migrating BMSCs with the injured tissue. Therefore, the number of homed stem cells very possibly determines the degree to which the ischemic tissue is repaired. Traditional Chinese medicines have a selective medicinal targeting effect, which can be applied to promote the homing of BMSCs. For example, Chinese medicines such as Bing Pian (Borneolium), She Xiang (Moschus), Shi Chang Pu (Rhizome Acori Graminei) etc., which restore consciousness and induce resuscitation, are highly fat-soluble and are of very low molecular weight. Such compounds can easily permeate the BBB to rapidly enter the brain, and have double-directional permeability across the BBB. They can also improve the function of the BBB and protect brain tissues by guiding other medical compositions to the target point. In addition to permeating the BBB themselves, they can also promote permeation of the BBB by other drugs to increase the therapeutic effect. Therefore, transplantation of BMSCs combined with Chinese medicines that restore consciousness and induce resuscitation may help BMSCs permeate the BBB. Another advantage of this medicinal targeting effect is the guidance of the BMSCs towards the injured brain tissue.

Synergistic therapeutic effects of drugs and BMSC differentiation

BMSCs are multipotent and can differentiate into osteoblasts, chondroblasts, muscle tendon, adipose cells, neurons and astrocytes etc., under appropriate conditions. This characteristic of BMSCs correlates highly with the TCM theory of the essence of life. Through tissue engineering studies using stem cells, researchers are studying the formation and regeneration of tissues and organs. This indicates that stem cells are the essence of human life because they can regenerate various human tissues and they conform to the basic property of maintaining living organs. Therefore, transplantation of exogenous BMSCs reflects the TCM method of replenishment essence in ischemic stroke treatment, and Chinese medicine provides a synergistic therapeutic effect that can promote essence by differentiating and repairing the injured brain marrow. It has been reported that Chinese medicines, including Danshen Injection, polypeptide of musk, total saponin of notoginseng, cryptotanshinone, Shenghuang Liquid, Astragalus Root, tortoise plastron liquid, Huangqi Injection, Renshen Injection, Danggui Injection, Naoxinshu Oral Liquid and Renshen Wang Jiang Oral Liquid, etc., can induce BMSCs to differentiate into neuron-like cells in vitro. Chinese medicines may therefore contribute to neogenesis by stimulating the differentiation and repair of injured nervous tissue. However, neogenesis not only promotes nervous tissue repair but also angiogenesis to provide a rich blood supply to the nervous tissue following ischemic stroke. Over-expression of SDF-1 in the local ischemic tissue was shown to increase the migration of BMSCs from peripheral blood and mediate the homing of endothelial progenitor cells to the ischemic tissue, where they induced angiogenesis and further tissue repair. Thus, the synergistic therapeutic effect of Chinese medicines for promoting angiogenesis in the ischemic zone may further improve neogenesis. It can be seen from the above discussion that the combination of Chinese medicine with transplantation of exogenous BMSCs may be potentially advantageous for removing blood stasis and stimulating neogenesis during treatment of ischemic stroke. Chinese medicines and exogenous BMSCs provide complementary functions for the removal of blood stasis and stimulation of neogenesis. Therefore, a combination of Chinese medicine and transplantation of exogenous BM-
SCs may be particularly suited to ischemic stroke treatment.

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