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Amniotic membrane mesenchymal stromal cell-derived secretome in the treatment of acute ischemic stroke: A case report

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Abstract

BACKGROUND

Stroke is the second and third leading cause of death and disability. To date, no definitive treatment can repair lost brain function. Recently, various preclinical researches has been reported on mesenchymal stromal cells (MSCs) and their derivatives about their potential as alternative therapies for stroke.

CASE SUMMARY

A 45-year-old female suffered an acute stroke, which led to paralysis in the left upper and lower limbs. Amniotic membrane MSCs-derived secretome (MSC-secretome) was intravenously transplanted to the patient four times with a week interval. MSC-secretome-regulated Treg cells, investigated the beneficial effects. The clinical improvement of this patient was accompanied by an increased frequency of Tregs after transplantation.

CONCLUSION

Intravenous administration of MSC-secretome potentially treats patients who suffer from acute ischemic stroke.

INTRODUCTION

Acute stroke is the second and third leading cause of death and disability, respectively, worldwide[1]. Although thrombolytic therapy is the only stroke treatment available, it can only be applied to a certain group of individuals. Additionally, dead neurons are not capable of regeneration. There is no long-term solution for recovering lost brain function[2]. Mesenchymal stromal cell (MSC) therapy has emerged as a promising therapeutic option for stroke as MSCs can migrate to lesions, secrete neurotrophic factors, reduce inflammation, and promote rehabilitation, thereby minimizing damage[3]. Neonatal birth-associated tissues, such as the umbilical cord (UC) and amniotic membrane (AM), are advantageous sources of MSCs because of their abundant availability, ease of collection, and lack of ethical restrictions[4]. We have developed a serum-free defined medium by the placing of all serum components with synthetic alternatives for deriving of clinical-grade UC-MSCs[5], finding that this serum-free medium enhanced the immunosuppressive effects of UC-MSCs[6]. Preliminary clinical studies using UC-MSCs to treat stroke have verified their beneficial effects and safety [2]. In recent years, preclinical and clinical studies. Our findings further demonstrated that AM-MSCs in a serum-free medium are more potent immunosuppressors than UC-MSCs[7], suggesting the usefulness of AM-MSCs for the treating of stroke. It has been found that the beneficial effects of MSCs are mediated mainly by the components of their secretome and, thus, the MSC-secretome is currently being studied in several clinical contexts, using either MSC-conditioned medium or purified MSC-derived extracellular vesicles to modulate tissue responses to a wide array of injuries[8]. There are significant benefits to using the MSC-secretome over MSCs in handling, safety, and the potential for standardization[9,10]. Our previous research confirmed that MSCs' and MSC-secretome immunomodulatory functions are similar[11]. These positive outcomes have led to clinical trials assessing the safety of the MSC-secretome for applications in the treatment of stroke, as well as the emergence of multiple commercial MSC-secretome sources marketed for topical application in cosmetic medicine[12,13]. In this article, we report the first case of a study describing a patient suffering from stroke who was treated with four infusions of allogeneic MSC-secretome.

CASE PRESENTATION

Chief complaints

On 2021-03-13, a 45-year-old female patient afflicted by stroke sought consultation at our medical facility. She presented with hemiplegia affecting the musculature of the upper and lower left extremities.

History of present illness

While moving around at home, the patient acquired slurred speech, numbness, weakness in the left limb, impairment in the left hand, and inability to walk independently.

History of past illness

The patient had a documented medical background of hypertension spanning a duration of nine years，which was treated with oral "nifedipine," although the blood pressure was not monitored regularly. The patient also denied a history of diabetes, cerebral hemorrhage, and coronary atherosclerosis.

Personal and family history

No special notes.

Physical examination

The patient exhibited paralysis in the upper and lower left extremities, resulting in ambulatory incapacitation and compromised speech function. The severity of stroke was evaluated based on the National Institute of Health Stroke Scale (NIHSS). At her first visit, the score was 12. The activities of daily living scale was evaluated based on the Barthel index (BI). The score was 30.

Laboratory examinations

The patient's complete blood count, coagulation, liver and kidney function, and electrolytes were generally normal following admission. The erythrocyte sedimentation rate was 9 mm/h, glycosylated hemoglobin 5.50%, triglycerides 1.10 mmol/L, total cholesterol 5.07 mmol/L, lipoprotein (a) 69.40 nmol/L, homocysteine 18.30 μmol/L, high-density lipoprotein cholesterol 1.18 mmol/L, LDL cholesterol 3.69 mmol/L, and glucose 7.09 mmol/L.

Imaging examinations

Computed tomography (CT) of the patient's head showed multiple lacunar cerebral infarctions with ischemic changes in the white matter. Diffusion-weighted imaging showed a new infarct focus in the paraventricular nucleus on the right side. Carotid ultrasound revealed several carotid plaques, whereas vertebral artery ultrasound indicated no abnormalities. The electrocardiogram showed normal sinus rhythm.

FINAL DIAGNOSIS

The diagnosis of acute ischemic stroke was established by analyzing the patient's history of symptoms, blood tests, and brain CT images. The categorization of stroke severity was determined as moderate to severe, as inferred from the NIHSS scoring.

TREATMENT

AM-MSC isolation, culture, and characterization

All human placental samples were collected from healthy, full-term, uncomplicated pregnancy. Prior to participation, all subjects provided informed consent, and the research protocol received formal approval from the Ethics Committee of the Affiliated Hospital of Chifeng College (No: 2021-033). Human AM-MSCs were collected as previously described[7]. In brief, the AM was mechanically peeled off from the placenta, washed with phosphate-buffered saline, and minced into approximately 1 mm^3 × 1 mm^3 pieces. The AM was incubated in 0.25% trypsin solution for 60 min at 37°C, followed by incubation in 200 U/mL collagenase solution for 60 min at 37°C. The released cells were

plated for five days in a serum-free defined medium developed in our laboratory, and removed to unattached cells. The formulation of serum-free defined medium including basal medium and xeno-free defined supplement was showed in our previous articles[14], and serum-free defined medium was prepared in our laboratory.

The adherent cells were washed and cultured at 37°C in 5% CO_2. Change for fresh medium twice per week. When reaching eighty percent confluence, the cells were passaged at 3000 cells/cm² (Figure 1). The AM-MSCs were characterized by their fibroblast-like morphology, the presence of distinct surface markers ($CD29^+$, $CD44^+$, $CD73^+$, $CD90^+$, $CD105^+$, $CD14^-$, $CD19^-$, $CD34^-$, $CD45^-$, and HLA-DR$^-$) and their potency to differentiate into osteoblasts, chondrocytes, or adipocytes in specific induction media (Figure 2A and B). The absence of contaminating pathogenic microorganisms and endotoxin release (< 0.5 EU/mL) from the cells was confirmed as described previously[5].

Preparation of the MSC secretome

MSC secretome was prepared from Beijing Protercell Biotechnology Co., Ltd and the detailed process of manufacture and quality control were presented as follows: Briefly, AM-MSCs at the fifth passage were cultured until 80% confluent, carefully cleaned, and seeded in serum-free Iscove's Modified Dulbecco's Medium to prepare the MSC secretome The conditioned medium was collected after 48 h, centrifuged at 5000 × g for 15 min to remove cells and cell debris, and passed through a 0.22-μm filter. The filtered media were concentrated by centrifugation using ultrafiltration units (Millipore, Bedford, MA, United States) with a 3-kDa cutoff. The protein concentration in the concentrated medium was measured using a BCA Protein Assay Kit (Neobioscience, China) and enzyme-linked immunosorbent assay kits (Neobioscience) were used to measure the contents of secreted cytokines, including serine protease inhibitor clade E member 1 (SERPINE1), insulin-like growth factor binding protein 4 (IGFBP4), interleukin-6 (IL-6), tissue inhibitors of metalloproteinase (TIMP)-1, TIMP-2, angiopoietin-like 4 (ANGPTL4), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and transforming growth factor-beta 1 (TGF-β1). Media were stored at −80℃ until use. The

average protein concentration for the entire secretome was 2.5 mg/mL. Nine molecules were detected in the secretome samples (Figure 2C), of which both SERPINE1 and IGFBP4 were secreted at amounts higher than 500 pg/mL (SERPINE1, 812; IGFBP4, 710). Five proteins were present at concentrations between 100 and 500 pg/mL (IL-6, 495; TIMP1, 307; TIMP2, 303; ANGPTL4, 254; HGF, 135) while two were between 100 and 10 pg/mL (VEGF, 63; TGF-β1, 28).

Treatment of the patient

One week after the stroke onset, the MSC secretome (50 mg of protein in 100 mL of 0.9% sodium chloride injection) were administered intravenously to the patient. The same process was performed four times with a one-week interval. The patient was discharged two days after the transfusion, with follow-up visits at 1, 3, 6, and 12 months to assess the NIHSS and BI scores and the proportion of peripheral regulatory T (Treg) cells (Figure 1). No other drugs or physiotherapy were used during treatment and follow-up monitoring.

OUTCOME AND FOLLOW-UP

The patient's neurological function improved gradually after MSC-secretome transfusion with significant improvement of the weakness in the left limbs. Moreover, the patient's mobility exhibited significant enhancement, encompassing not only basic ambulation and limb motility, but also encompassing proficiency in intricate activities and prolonged periods of upright stance. The NIHSS score decreased from 12 at baseline (before transplantation) to 3 at 12 mo after transplantation (Figure 3A). Moreover, the BI score increased from 30 to 60 (Figure 3B).

During treatment and follow-up monitoring, the patient observed or reported no adverse reactions related to mobility. In addition, the patient manifested an absence of symptoms such as fever, chills, or nausea that frequently occur in patients who receive MSC-secretome transplantation. The patients' peripheral Treg levels were analyzed before and after the treatments. This showed that the proportion of $CD4^+CD25^+127^{low/-}$

Tregs in the $CD4^+$ T cell population increased gradually over the 12 mo after transplantation (Figure 4). These findings suggest that the secretome transplanting changed Treg levels, producing advantageous benefits. After receiving MSC-secretome therapy, the patient's clinical condition improved along with an increase in the number of Tregs.

DISCUSSION

AM-MSCs possess strong immunomodulatory functions and promote tissue repair and regeneration through paracrine-soluble factors[15]. There are many advantages to using the MSC secretome for stroke recovery compared to employing the original MSCs[13]. First, treatment with the MSC secretome avoids the safety concerns associated with cellular transplantation, such as uncontrolled cell proliferation, differentiation, and division. Second, the MSC secretome does not have the problems of immune rejection and tumor promotion. Third, the MSC secretome is easier to store and transport than cells. In addition, the MSC-secretome has an inherent ability to home in on tissue injury sites due to the small size of the vesicles and their lipid bilayer membrane structure and, in particular, can cross the blood-brain barrier[16]. In this study, we describe a case of stroke treated with cell-free MSC-derived therapy using the MSC secretome. The patient received four transfusions of the MSC secretome at one-week intervals. The patient had not received any other form of therapy during or after the MSC-secretome treatment. At 12 mo following treatment, the patient's NIHSS score dropped from 16 to 3, suggesting the return of motor function, while her BI score increased from 30 to 60, confirming significant improvements in her quality of life.

A previous study identified 200 factors released by AM-MSCs[15]. The present study analyzed the AM-MSC secretome to determine the presence of trophic, chemotactic, and immunomodulatory factors. The results revealed an abundance of cytokines in the secretome, including SERPINE1, IGFBP4, IL-6, TIMP-1, TIMP-2, ANGPTL4, VEGF, HGF, and TGF-β1. The vascular trophic factors (VEGF, HGF, IGFBP4, and ANGPTL4) are important effector molecules that promote tissue repair, not only restoring the blood

supply in ischemic tissues but also providing neuroprotective effects in acute stroke[17,18]. Recent studies have confirmed that the chemotactic factors SERPINE1, TIMP-1, and TIMP-2 play crucial roles in peripheral neutrophil infiltration, which may reduce the effects of neuronal injury induced by ischemia-reperfusion[19]. This finding shows that SERPINE1, abundant in the MSC secretome, may play an important role in the acute stage of ischemic stroke. More importantly, IL-6, HGF, and TGF-β1 are molecules associated with immune function and could contribute to immunomodulatory properties. It has been confirmed that these molecules either reduce inflammation[20] or have a pivotal role in the positive regulation of the immune system[21]. Further investigations are required into the specific cytokine (s) responsible for the most significant improvements in stroke are required.

As an important subpopulation of immunosuppressive T cells, regulatory T cells (Tregs) are involved in maintaining immune homeostasis and regulating immune-modulatory responses in the pathological process of ischemic stroke[22,23]. The proportion of Tregs in this patient was found to be below normal values, consistent with previous findings[24]. Immunosuppression by the MSC secretome has been revealed to contribute substantially to the efficacy of stroke treatment. This is associated with an upregulation of anti-inflammatory Tregs[25], which is consistent with the present patient's follow-up results. Recent studies have also shown that Tregs may participate in the recovery of ischemic stroke patients[26,27], and *ex vivo* expansion of Tregs and their products could suggest therapeutic prospects aimed at safeguarding neurons against stroke and mitigating neuroinflammatory disorders [28,29]. These findings support the possibility that increased peripheral Treg levels may be associated with the therapeutic benefits observed in this patient.

CONCLUSION

In conclusion, our study indicated the feasibility of using cell-free MSC-secretome therapy for stroke. This is the first human case treated with intravenous transplantation of the MSC-secretome. The beneficial effects may be associated with increased peripheral

Treg levels. These and additional studies' findings might eventually result in the development of novel secretome-based treatments for stroke alongside the implementation of the currently used MSCs. However, more research involving a larger sample size of patients is required.

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