Umbilical cord blood mesenchymal stem cell transplantation for treatment of a child with spinal muscular atrophy

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Abstract

BACKGROUND: Many animal and clinical studies have reported that the safe and effective usage of umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs) transplantation for treatment of neurological genetic diseases.

OBJECTIVE: To investigate the feasibility and effect of UCB-MSCs transplantation in the treatment of spinal muscular atrophy (SMA).

METHODS: A child admitted at January 2010 had been confirmed as having SMA, and drug and rehabilitation therapies were invalid. Then, the child received UCB-MSCs transplantation *via* the first intravenous infusion and three times of subarachnoid injection, once a week, $(4–6)\times10^7$ cells once and four times as a course. Neurological physical examination, biochemical test, muscle enzymes detection, FIM scoring and electromyography (EMG) examination were conducted.

RESULTS AND CONCLUSION: Compared with prior to transplantation, the level of muscle enzymes decreased, FIM scores were increased from 68 to 93 points, EMG results showed that the motor units with re-contraction in each 10.0 ms were increased that the motor function was improved, the lower extremity muscle strength elevated, and the self-care ability was improved in the SMA child at 6 months after transplantation. During the 10-month follow-up, the child had no adverse effects. It is indicated that UCB-MSCs transplantation is effective to treat SMA, and the neurological function has a remarkable restoration.

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INTRODUCTION

Spinal muscular atrophy (SMA) is an autosomal recessive disease characterized by degeneration of motor neurons. SMA is the second commonest genetic cause of death in childhood with an incidence of 1 in 6 000–10 000[1]. Clinical manifestations are progressive and symmetrical muscle weakness and atrophy, which is severer in the proximal than the distal, as well as severer in the lower limb than the upper limb[2]. Currently, there are still no available effective treatments.

With the emergence of regenerative medicine, stem cell transplantation has become a hot research for many medical workers. Stem cell transplantation can replace damaged nerve cells, promote cell structure and function reconstruction, which is a new idea and approach in the treatment of neurological diseases[3-4]. In China, stem cell transplantation in the treatment of neurological genetic diseases has been reported a lot, but its treatment of SMA has not been reported in the world.

A child with SMA-Ⅱ receiving umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs) at Taihe Hospital of Hubei Medical College from 2009–12–23 to 2010–01–15 was included in this study to explore the effectiveness and effect of UCB-MSCs transplantation on SMA.

CASE INTRODUCTION

Medical history

A boy, 2 years and 2 months old, was admitted due to weak walking and walking swinging on January 15, 2010. The child began to walk when he was 18 months old, but he was unwilling to walk, could not stand from the pedal position, walk weakly, and was easy to fall down, with the performance of the chest facing straight forward, swinging, waddling gait, slow moving, and inability to lifting the toe from the ground. In addition, the boy could walk 2.0 to 3.0 m once, and did not present with remarkable upper limb weakness. Previous history: the boy appeared with motor function retardation of the lower limbs when he was 6 months old. Family history: his parents were healthy who were of non-consanguineous marriage, and there was no similar case in the family history. The child received neurotrophic drug treatment assisted with rehabilitation training at the age of 18 months old. However, the symptoms were not relieved and progressively aggravated.

Neurological examination

The child presented with conscious mind, clear speech, normal intelligence, no abnormalities of the cranial nerves, poor muscle tone of the lower limbs, disappearance of tendon reflexes, negative pathological reflex, no sensory dysfunction, thigh muscle atrophy, pseudo-hypertrophy of the gastrocnemius of the lower limbs, muscle strength of grade 2 at the proximal end and grade 4 at the distal end, normal muscle strength of the upper limbs, waist and spine lordosis when standing, swinging when walking, and waddling gait. The both hand of the child trembled obviously, and the child also showed a visible muscle twitching of the tongue and limb-girdle muscle. Gower sign was positive. The score of functional independence measurement (FIM) was 68 points. The results of laboratory tests showed that lactate dehydrogenase 388 IU/L (normal 72 to 182), creatine phosphokinase 148 IU/L (normal 25 to 170), creatine kinase MB 48 IU/L (normal 0 to 17), a-hydroxybutyrate dehydrogenase 318 IU/L (normal

72 to 182). The electrocardiogram (ECG) measurement was normal as well as thoracic and lumbar MRI. Electromyogram (EMG) examination detected neurogenic damage, the fibrillation and positive phase potential of the bilateral anterior tibial muscle, right lateral vastus medialis, and left first dorsal interosseous muscle, light contraction motor units with large potential, 4 or 5 motor units presenting with re-contraction in each 10.0 ms. The polyacrylamide gel electrophoresis genetic testing performed in the Xiangya Hospital of Hunan showed that the pure absence of SMNt gene exons 7, 8.

Clinical diagnosis

The child was confirmed as having SMA Ⅱ.

UCB-MSCs source, preparation and therapeutic approaches

In 2010, Hubei Health Bureau approved UCB-MSCs clinical technology for the third class medical technology in the Taihe Hospital of Hubei Medical College, which was allowed for clinical application. The treatment program was approved by the Hospital Medical Ethics Committee, and the informed consent was obtained from the child's guardians.

Human umbilical cord blood collection: The cord blood samples were collected from healthy pregnant women who had no pregnancy complications, delivered at 37–40 gestational weeks and agreed voluntary contribution for scientific research and clinical treatment. Puncture under sterile conditions was used for collection of healthy, full-term fetal umbilical vein with heparin blood collection bags, each bag containing 50–100 mL cord blood. The cord blood samples underwent HIV, syphilis antibody, hepatitis markers 5, Torch5 tests.

UCB-MSCs isolation and purification: The blood samples were mixed with hydroxyethyl starch at a ratio of 2:1 and placed into 150 mL sterile plastic bottle, and stand 90–120 minutes for sedimentation of red blood cells. The cell supernatant was added carefully at a ratio of 1:1 to the lymphocyte separation medium with a relative density of 1.077, centrifuged at 2 000 r/min for 30 minutes to drawn the white film layer; 5-time volume of normal saline was added followed by 10 minutes centrifugation at 1 000 r/min, rinse 3 times to remove the supernatant. Then, mononuclear cells suspension was prepared and inoculated in serum-free medium; the medium was changed after 3 days to remove non-adherent cells. After that, the medium was changed every 3 days. When cells grew to 80% confluence, 1:1 trypsin + EDTA digestion was done, and uniform long spindle-shaped cells were seen in the culture flask after third or fourth passage, which were identified as UCB-MSCs by flow cytometry. The UCB-MSCs suspension was prepared for intravenous infusion of 50 mL and subarachnoid injection of 1 mL, $(4–6)\times10^7$ cells once.

Therapeutic approaches: First, peripheral intravenous infusion of UCB-MSCs was conducted followed by the subarachnoid injection 3 times. Three days prior to the treatment, the child was required to have normal temperature, no skin damage, no signs of infection. Clinical symptoms, body signs, FIM score, EMG and clinical examination served as outcome measures, which were observed before treatment, immediately and 6 months after treatment.

Disease outcome

Re-examination at 6 months after treatment showed that the child could walk 15–20 m with normal walking willingness and reduced fatigue feeling, and his indoor activities were not limited. When walking, the boy also swung and the chest faced straight forward, presenting with waddling gait. However, the child could go upstairs with the help of fence, stand from the pedal position supported by other things, and lift off his toes from the ground. Neurological examination showed reduced muscle tone of the both lower extremities, disappearance of tendon reflexes, full thigh muscles, non-improved pseudo-hypertrophy of the gastrocnemius of the lower limbs, muscle strength of grade 3 or 4 at the proximal end and grade 5 at the distal end, both hand tremor, unobvious tremble of the limb girdle, and slightly tongue tremor (significantly relieved compared with the previous). Gower sign was positive. The FIM score was 93 points. The results of laboratory tests showed that lactate dehydrogenase 241 IU/L, creatine phosphokinase 145 IU/L, creatine kinase MB 37 IU/L, a-hydroxybutyrate dehydrogenase 191 IU/L. The EMG examination detected neurogenic damage, the fibrillation and positive phase potential of the bilateral anterior tibial muscle, right lateral vastus medialis, and left first dorsal interosseous muscle, light contraction motor units with large potential, 7 or 8 motor units presenting with re-contraction in each 10.0 ms. During the 10-month postoperative follow-up, the clinical symptoms were relieved progressively.

Intervention comment

After treatment, no fever, infections, rashes and other complications occurred; at 6 months after treatment, clinical symptoms and relevant laboratory examinations showed significant improvement in neurological function; the follow-up of 10 months showed continuous improvement of clinical symptoms and no adverse reactions.

DISCUSSION

SMA is a neurological genetic disease, and has been subdivided into three clinical types according to the age of onset and disease course: type Ⅰ, Werding-Hofman atrophy, is characterized by the onset of severe symptoms within 6 months after birth and the inability to sit or stand. Fatal respiratory failure often occurs in the first 2 years. Type Ⅱ SMA is a moderate SMA, characterized by the onset of symptoms before 18 months of age. Children can sit but not walk unaided, and they can survive beyond 2 years. Children with type Ⅲ SMA (Wohlfart-Kugelberg-Welander syndrome) are able to sit and walk independently and survive into adulthood. In recent years, molecular genetic studies have shown that SMA is induced by the decrease of the survival of motor neuron gene (SMN) protein expression causing the degeneration of the anterior horn cells of the spinal cord and eventually resulting in muscle atrophy and paralysis[5]. Although the exact location of virulence genes is a new drawn for patients who has no effective treatment for SMA, the clinical trials of nearly 10 drugs have not obtained positive or preferable results. Domestic and foreign scholars believe that gene therapy and stem cell therapy are two potential

treatment strategies for SMA. The gene therapy has harvested some encouraging results in animal experiments, but its clinical application still needs a long-term study. Cell transplantation is an important approach to treat central nervous system diseases. In 2000, Erices *et al*[6] discovered MSCs during in vitro culture of cord blood mononuclear cells, and then scholars confirmed the presence of UCB-MSCs[7-8]. Jurga *et al*[9] reported the functional properties of human UCB-MSCs under three-dimensional culture: expression of nestin, GFAP, MAP2, and production of action potentials, *etc*. Seo *et al*[10] reported that during UCB-MSCs transplantation for treatment of mice Pick disease, UCB-MSCs can inhibit cell apoptosis associated with inflammatory reactions to reduce the loss of Purkinje neurons, which is associated with the PI3K/AKT and JAK2/STAT3 signaling pathways. Newcomb *et al*[4] found that the intravenous injection of UCB-MSCs can reduce neuronal apoptosis and inhibit inflammatory responses to protect t neurons and reduce the neurological deficit scores following cerebral infarction in a cerebral ischemia model. UCB-MSCs can be induced to differentiate into neurons and glial cells, and secrete a variety of growth factors under the micro-environment of the central nervous system, or produce endogenous factors through stimulating the injury site to promote the repair of damaged tissue and reduce the number of apoptotic cells. For this reason, UCB-MSCs are considered as ideal seed cells for stem cell transplantation in the treatment of neurological diseases[9-10]. Domestic scholars have conducted a series of basic research about UCB-MSCs in the treatment of neurological diseases[11-12]. ①Flow cytometry was used to detect Nestin expression changes in patients with neurological diseases before and after UCB-MSCs transplantation. At 1–2 weeks after UCB-MSCs subarachnoid transplantation, Nestin expression of the original naive/stem cells in the cerebrospinal fluid increased strongly, and Nestin-positive cell ratio significantly increased, indicating that UCB-MSCs *in vivo* are certainly able to differentiate into neural stem cells. ②Transplanted human UCB-MSCs could survive in Parkinson's disease rats for a long term and migrate along certain routes to differentiate into nerve cells, directly providing a more objective basis for clinical treatment of neurological diseases with the UCB-MSCs transplantation. In addition, there are also attempts to realize relevant clinical applications[13-14].

①FIM improvement after treatment: FIM scoring on amyotrophic lateral sclerosis, motor neuron disease, hereditary spinocerebellar ataxia and other diseases was significantly improved after UCB-MSCs treatment, and the patient's daily living skills were improved significantly[13].

②UCB-MSCs could promote neuronal repair *via* the immune regulation. Detection results of T cell subsets showed that there was a downward trend in total T cells, indicating that UCB-MSCs transplantation has a self-regulation role in cellular immunity, creating favorable conditions for short-term repair of impaired neurons[13].

③The cerebral blood flow volume in the basal ganglia was increased in patients with Parkinson's disease receiving UCB-MSCs transplantation, which may be related with that MSCs is a major component of new blood vessels at the injured site, and can be differentiated into vascular endothelial cells and extracellular matrix, helping to nerve protection and angiogenesis promotion[14].

④Good security: UCB-MSCs transplantation for treatment of neurological diseases is mainly *via* the intravenous plus subarachnoid injection methods. It is reported that only a few patients presented with fever, headache and other mild side effects after UCB-MSCs transplantation, and these symptoms were all relieved within 3 days, and did not affect the daily life, showing good safety.

In the present study, the child could not walk independently before 18 months of age, presented with symmetrical muscle weakness of the both lower extremities, mainly in the proximal limbs; bundle tremor, severer in the lower limbs than the upper limbs; walking swinging due to pelvic girdle muscle weakness, showing a progressive increase; pseudo-hypertrophy of the gastrocnemius of the lower limbs; positive Gower sign; tongue tremor. The level of serum muscle enzymes was slightly elevated, and EMG showed a typical neurogenic damage. The results of genetic test showed SMNt loss exon 7, 8. According to the above findings, the child was diagnosed as having type Ⅱ SMA, and the genetic test results were the most important evidence to confirm type Ⅱ SMA[15]. After confirmed diagnosis, the 6-month routine drugs and physical therapy were invalid. With the approval of the hospital medical ethics committee and informed consent from the guardian of the child, UCB-MSCs transplantation was conducted via the first intravenous infusion and three times of subarachnoid injection as one course, in reference with the domestic literatures addressing UCB-MSCs transplantation for treatment of amyotrophic lateral sclerosis, Parkinson's disease, multiple system atrophy and other diseases[13-14,16]. UCB-MSCs transplantation needs a relatively lower requirement about the matching between the human leucocyte antigens of donors and recipients. CD3 and CD4 are relatively immature, antigen expression and function activity are lower, and the incidence of graft versus host disease is also low[13-14,16]. Based on clinical applications reported in the literature, pre-treatment tissue matching is unnecessary. No uniform standards about the number of transplanted MSCs are reported. We adopted $(4–6)\times10^7$ cells once, which are the amount of MSCs harvested following isolation, extraction and culture from 100 mL umbilical cord blood of a pregnant woman. After UCB-MSCs transplantation, the child was good in general conditions, had no fever, rash and o other immune rejection occurred. Re-examination at 6 months after UCB-MSCs transplantation showed increased muscle strength, higher FIM scores, improved capacity to daily living, reduced muscle enzymes level, and EMG examination showed increased number of motor units. After 10 months, these symptoms were progressively relieved, and no recurrence occurred, indicating that UCB-MSCs treatment prevents the disease progression and obviously restores the neurological function in a short term in this child with type Ⅱ SMA.

In short, UCB-MSCs transplantation has no serious adverse reactions, and it is initially safe. The use of UCB-MSCs transplantation for treatment of SMA has not been reported in foreign countries. Although the short-term outcomes of this child is satisfactory, there are some limitations, such as the case number and follow-up duration, this disease cannot be

replicated in animal models, lack of rigorous clinical controlled trials and animal experimental studies. In addition, this new technology must be carried out after the disease is confirmed and patients' safety is ensured under the approval of the Ethics Committee and informed consent of family members of patients. A series of scientific and objective criteria for efficacy evaluation should be established as well as a long-term follow-up for curative effects and side effects. Without a doubt, SMA is a hereditary neurological disease that ultimately relies on the clinical application of gene therapy. However, at the present stage, UCB-MSCs transplantation is safe and can significantly improve neurological function and quality of life in SMA children, which is worth further studies.

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脐血间充质干细胞移植治疗儿童型脊肌萎缩症 1 例

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摘要

背景：国内外已有实验动物和临床应用脐血间充质干细胞移植治疗神经系统遗传性疾病安全、有效的诸多报道。

目的：探讨脐血间充质干细胞移植治疗儿童进行性脊髓性肌肉萎缩症的可行性及效果。

方法：2010-01 收治 1 例确诊为儿童进行性脊髓性肌肉萎缩症患儿，经药物及康复治疗无效，行脐血间充质干细胞移植治疗。移植途径采取首次静脉输注，后 3 次蛛网膜下腔注入，1 次/周，每次细胞数量达(4~6)×10^7 个，4 次为 1 个疗程。治疗前和治疗后半年均需完善神经系统体检、实验室检查、肌酶、FIM 评分、肌电图等。

结果与结论：移植 6 个月与移植前比较，肌酶下降，FIM 评分由 68 分提高到 93 分，肌电图检查重收缩每 10.0 ms 所检肌运动单位增加两三个，双下肢肌力增加，生活自理能力改善。随访 10 个月，患者未出现明显的不良反应。提示脐血间充质干细胞移植治疗该例儿童进行性脊髓性肌肉萎缩症患儿有效，其神经功能恢复明显。

关键词：脐血间充质干细胞；细胞移植；脊髓性肌萎缩症；儿童；神经功能
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伦理批准：本例接受脐血间充质干细胞移植治疗患儿，治疗前由其法定监护人签署了“脐血间充质干细胞移植治疗知情同意书”，该治疗方案获得了医院伦理委员会批准；健康正常分娩孕妇知情同意将脐血样本用于此研究。

本文创新性：国内外学者认为基因治疗和干细胞治疗是两项对儿童型脊髓性肌萎缩症都有潜力的治疗策略，但基因治疗尚在动物实验阶段，干细胞治疗该病也未见报道。国内有脐血间充质干细胞移植治疗神经系统遗传性疾病安全、有效的报道，但针对儿童型脊髓性肌萎缩症没有尝试。该文证实脐血间充质干细胞移植治疗儿童型脊髓性肌萎缩症有效、安全，属应用创新。