Intravenous infusion umbilical cord-derived mesenchymal stem cell in primary immune thrombocytopenia: A two-year follow-up

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Abstract. Four patients with chronic refractory immune thrombocytopenic purpura (ITP) received human umbilical cord-derived mesenchymal stem cells (hUC-MSCs). The hUC-MSC dose was 5x10^7 to 1x10^8. Complete remission (CR) was achieved in three patients in 12 months and one patient in 24 months. Three patients received the second hUC-MSC transplantation with the same dose. The median time between hUC-MSC transplantation and response was 12.5 days (range, 7-16). There were no severe adverse events during and post hUC-MSC transplantation. During follow-up (median, 17 months; range, 13-24) no other immuno suppressive drugs were used post-first hUC-MSCs transplantation. In conclusion, hUC-MSC transplantation is a reasonable salvage treatment in chronic refractory ITP. Prospective randomized large-scale clinical trials are needed to further elucidate the efficacy of hUC-MSCs transplantation therapy on ITP.

Introduction

Chronic immune thrombocytopenic purpura (ITP) is an auto-immune disorder characterized by an elevated rate of platelet destruction and persistent thrombocytopenia (1,2). Patients with chronic refractory ITP have the highest risk of death and disease-related or therapy-related complications (3,4). Despite intense research efforts and large multicenter clinical trials, the optimal treatment for patients with chronic ITP in clinical practice remains to be determined (5).

The number of studies focusing on the therapeutic potentials of mesenchymal stem cells (MSCs) in experimental models and clinic trials are growing. One of the reasons for this growing interest can be explained by the fact that MSCs are assumed to be effective biological tools to treat degenerative diseases. In previous studies, we grafted MSCs derived from human umbilical cord-derived MSCs (hUC-MSCs) to treat non-union in rats and humans (6-8). Our results demonstrated the safety as well as the efficiency of osteoblastic differentiation of hUC-MSCs. In the present study, we describe our experience using hUC-MSCs to treat patients with chronic refractory ITP.

Materials and methods

Basic principles and ethical considerations. The protocol of the present study was approved by the Institutional Review Board and the Ethics Committee of Siping Hospital of China Medical University (Beijing, China). The trial was conducted in compliance with current Good Clinical Practice standards and in accordance with the principles set forth under the Declaration of Helsinki in 1989.

Confirmation of isolation and propagation of hUC-MSC. hUC-MSCs used in this trial were derived from two donated umbilical cords (UC) obtained from healthy mothers during routine term elective cesarean section births. Fully informed consent was obtained several weeks prior to delivery. hUC-MSCs were isolated and propagated, as previously described (6-8).

Patients. ITP was diagnosed in accordance with standard criteria and other causes of thrombocytopenia were excluded. Three adult patients with ITP having a platelet count <30x10^9/l that persisted for at least 3 months with an inadequate or transient response to multiple therapies were treated with hUC-MSCs. The patients were willing to sign an informed consent form where they agreed to be treated in the Siping...
Hospital of China Medical University. The general characteristics of the patients are presented in Table I.

**Intravenous infusion of hUC-MSCs.** hUC-MSCs (10 ml) with a cell density of 5x10^6 to 1x10^7/ml was given intravenously at a rate up to 12.5x10^6/min and flushed with 20 ml saline to ensure full cell dose delivery. Once the needle was fully withdrawn, the puncture site was wrapped with sterile dressing. Patients remained in the supine decubitus on the operation bed for another 30 min before off-bed activities and antibiotics were given to prevent infection. Patients' conditions were monitored (temperature, blood pressure, pulse and oxygen saturation) at 15, 30, 45 and 60 min, and then once every hour for a minimum of 4 h. They were discharged 24 h post-transplantation if they were not febrile and hemodynamically stable, with no signs of infection or any type of allergic reaction. Any abnormal reactions within 3 months were considered to be linked to transplantation.

**Measurement of platelet related parameters.** Platelet-related parameters were analyzed before the operation and at several time points post-transplantation using an automated blood cell counter model LH-750 (Beckman Coulter, Inc., Brea, CA, USA).

**Clinical and functional assessment.** i) Primary safety assessments included monitoring and recording of all the adverse events as well as the serious adverse events. The patients were monitored (temperature, blood pressure, pulse and oxygen saturation) at 15, 30, 45 and 60 min, and then once every hour for a minimum of 4 h. They were discharged 24 h post-transplantation if they were not febrile and hemodynamically stable, with no signs of infection or any type of allergic reaction. Any abnormal reactions within 3 months were considered to be linked to transplantation.

**Pharmacological therapy protocol.** Pharmacological therapy consisted of: i) inhaling high doses of steroids and prednisone (1 mg/kg, p.o., once daily); ii) vincristine (2 mg, once per month, i.v.); iii) intravenous immunoglobulins (γ globulin), 0.4 g/kg, once daily, i.p.; and iv) cyclosporine (3 mg/kg, p.o., once daily).

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| Characteristics          | Patient 1 | Patient 2 | Patient 3 | Patient 4 |
|--------------------------|-----------|-----------|-----------|-----------|
| Age (years)              | 26        | 49        | 54        | 50        |
| Gender                   | M         | F         | F         | F         |
| Duration of disease (months) | 43        | 71        | 62        | 120       |
| Previous treatments      | P, V, C, IVIg, S | P, V, C, IVIg | P, IVIg, C, De, S | P, IVIg, V |
| HUC-MSC transplantation times | 1        | 2         | 2         | 2         |

Platelet counts (x10^9/l)

| Before therapy | 8 | 9 | 5 | 3 |
| After therapy (2 weeks) | 56 | 94 | 103 | 56 |
| After therapy (3 months) | 80 | 96 | 105 | 59 |
| After therapy (6 months) | 82 | 101 | 118 | 61 |
| After therapy (12 months) | 189 | 84 | 234 | 116 |
| After therapy (24 months) | 134 |

Bleeding

**Before therapy**

| Skin, genitourinary bleeding | Skin | Epistaxis | Skin, genitourinary bleeding |
|------------------------------|------|-----------|-----------------------------|
| After therapy               | No   | No        | Mucosal                     |
| Time to response (days)     | 7    | 13        | 16                          |
| Time to maximum response (days) | 31 | 53        | 42                          |
| Overall response            | Yes  | Yes       | Yes                         |
| Response duration (months)  | Yes, 24 | Yes, 18 | Yes, 13                     |

*Platelet count measurements were carried out at the end of the following time points post hUC-MSC transplantation. *Major skin indicates diffuse ecchymosis; mucosal, intrabuccal hemorrhagic vesicles, or prolonged epistaxis; and intestinal and menorrhagia, gastrointestinal, and genitourinary bleeding, respectively. These four patients had a relapse within 13 months after the first hUC-MSC administration but responded to the second hUC-MSC treatment, and they all sustained response for >8 months. hUC-MSCs, human umbilical cord-derived mesenchymal stem cells; M, male; F, female; P, prednisone; V, vincristine; C, cyclosporin; IVIg, intravenous immunoglobulins; S, splenectomy; De, dexamethasone.
Statistical analysis. Statistical analyses were performed using SPSS 16.0 software (Chicago, IL, USA). Safety and exploratory efficacy secondary endpoints were observed for each patient against the baseline values. P<0.05 was considered statistically significant.

Results

Evaluation of hUC-MSCs. Cells derived from UC were observed 24 h after they were seeded (Fig. 1A), during the time that part of the round mononuclear cells was adherent. Three days after inoculation, small colonies of the adherent cells with typical fibroblast-shaped morphology were obtained (Fig. 1B). These primary cells reached monolayer confluence, after planting for 5-6 days, when they were passaged for the first time. Fifth passage cells were analyzed by flow cytometry and were strongly positive for CD105 and CD90, but negative for CD45 and HLA-DR (Fig. 1C and D).

Patient characteristics. Clinical characteristics of patients who participated in the present study are summarized in Table I. The patients were 3 females and 1 male, with an age range of 26-54 years (median, 44.75 years). Median duration of ITP before hUC-MSC transplantation was 74 months (range, 13-120 months) and the median number of prior treatments was 2 months (range, 1-3 months), which included splenectomy, prednisone, intravenous immune globulin, cyclosporine and vincristine. All the patients had a history of major bleeding and those episodes were often transient but recurrent. Major hemorrhagic events included genitourinary bleeding, diffuse ecchymosis and prolonged epistaxis.

Clinical therapeutic effect of hUC-MSCs. Results of hUC-MSC treatment are shown in Table I. Overall responses were reached in all the patients at the end of the second week after the hUC-MSCs had been administered. The patients achieved a platelet count of >50x10^9/l and 2 patients achieved a platelet count of >90x10^9/l. The median platelet count on treatment was 77.25x10^9/l (range, 56x10^9 to 10.3x10^10/l). The median time to response and the median time to maximum response were 12.5 days (range, 7-16 days) and 46 days (range, 31-53 days), respectively. One patient sustained response after a single course of hUC-MSCs without any further therapy during the follow-up. Major bleeding episodes did not occur. The remaining 3 patients (patients 2-4) had a relapse within 12 months after the first hUC-MSC administration but responded to the second hUC-MSC treatment. The time to the second response for patients 2, 3 and 4, was 13, 16 and 18 days, respectively, whereas the time to the second maximum response was 34, 38 and 43 days, respectively. All the patients achieved a sustained response of >10 months.

Safety outcomes. No serious or clinically significant side effects were observed during the entire study period. During
Prospective randomized clinical trials are needed to elucidate the efficacy of hUC-MSC transplantation therapy on ITP in the future.

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