Cytotherapy, 2017; 19: 194–199

International Society for Cellular Therapy
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Therapeutic potentials of umbilical cord–derived mesenchymal stromal cells for ischemic-type biliary lesions following liver transplantation

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Abstract

Background aims. Ischemic-type biliary lesions are severe, graft-threatening complications after orthotopic liver transplantation, and a novel and efficient therapeutic strategy is urgently needed. Due to the immunosuppressive and regenerative properties, mesenchymal stromal cells (MSCs) could be an interesting candidate. *Methods.* We initiated safety and efficacy of human umbilical cord–derived MSC (UC-MSC) transfusions for patients with ischemic-type biliary lesions after liver transplantation. From January 2013 to June 2014, 12 ischemic-type biliary lesions patients were recruited as the MSCs group in this phase I, prospective, single-center clinical study. Patients in this group received six doses of UC-MSCs (about 1.0×10^6 MSCs per kilogram body weight through peripheral intravenous infusion). The traditional therapeutic protocol was applied during October 2003 to December 2012 in 70 ischemic-type biliary lesions patients who were treated as the control group. Liver function tests, the need for interventional therapies and graft survival rate were chosen to evaluate the therapeutic efficacy of MSC treatment. Adverse events were closely monitored up to 2 years after MSC transfusions. *Results.* No significant MSC-related adverse events were observed during the trial. Compared with baseline, the levels of total bilirubin, γ-glutamyl transferase and alkaline phosphatase were decreased after UC-MSC treatment at week 20 and week 48. Interventional therapies were performed in 64.3% (45/70) of patients in the control group and 33.3% (4/12) of patients in the MSCs groups. MSC therapy significantly decreased the need for interventional therapies ($P = 0.046$). The 1- and 2-year graft survival rates were higher in the MSCs group (100% and 83.3%, respectively) than in the control group (72.9% and 68.6%, respectively). *Conclusions.* The UC-MSC transfusions are clinically safe and short-term favorable, which may become a novel treatment for patients with ischemic-type biliary lesions after liver transplantation.

Key Words: *ischemic-type biliary lesions, liver transplantation, mesenchymal stromal cells, umbilical cord*

Introduction

Donation after cardiac death is a major source of organs for liver transplantation worldwide. This kind of donation expands the donor pool [1–3], but also increases the rate of ischemic-type biliary lesions (ITBLs), which remain to be the most troublesome biliary complication and are the leading cause for graft loss after orthotopic liver transplantation (OLT). The incidence of ITBLs varies greatly in different studies, ranging from 1% to 26% [4–8]. This variation is probably due to the different sources of donor (donation after cardiac death or brain death), and partly due to different diagnostic interpretations. ITBLs are defined as nonanastomotic strictures and dilatations in the absence of other conditions such as hepatic artery stenosis or thrombosis, portal thrombosis, ABO incompatibility and primary sclerosing cholangitis. The therapy for ITBLs is not standardized, and varies due to medication treatment (ursodesoxycholic acid, antibiotic), endoscopic and percutaneous therapy and partial hepatectomy and/or choledochojejunostomy.

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(Received 24 July 2016; accepted 9 November 2016)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2016.11.005

However, ITBLs are frequently resistant to the traditional therapy, and retransplantation is the only option in up to 50% of the patients [9,10].

Mesenchymal stromal cells (MSCs) are characterized by the immunosuppressive and regenerative properties that make MSCs very attractive in treating immunologic diseases, including transplant rejection [11–19], and organ/tissue ischemic injury [20–27]. Considering the damage of peribiliary vascular plexus of biliary duct and immunologic injury are the main mechanisms of ITBLs, we hypothesized that MSCs transfusions may thus constitute a new therapeutic option for patients with ITBLs. To test this speculation, we transfused UC-MSCs six times to 12 recipients who developed ITBLs after OLT, aimed at confirming the safety and efficacy of UC-MSCs transfusions in ITBLs patients.

Methods

Patients

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. This study was the previous work of a clinical trial, which was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-Sen University (ClinicalTrials.gov, NCT02223897). From January 2013 to June 2014, 12 recipients developed ITBLs after OLT in the liver transplantation center of our hospital and were recruited in this trial. Seventy ITBL patients, who underwent OLT during October 2003 to December 2012, were treated as the control group. Detailed demographic and clinical characteristics of recipients are summarized in Table I. All patients who received MSCs treatment gave written informed consent in accordance with the institutional review board guidelines. The inclusion criteria were: (1) 18–70 years of age; (2) first liver transplantation; (3) confirmation of ITBLs with cholangiography; and (4) willing to participate in the study and must be able to give informed consent. The exclusion criteria were: (1) existence of systemic infection; (2) ABO incompatibility between the donor and recipient; (3) hepatic arterial thrombosis after surgery; (4) primary sclerosing cholangitis or hepatic malignant tumor as the primary disease; and (5) incomplete follow-up. The baseline data were collected after patient enrolled in the study and 1 week before UC-MSC transfusions.

Table I. Demographic and clinical characteristics of ITBLs patients in the two groups.

	Control (n = 70)	MSCs (n = 12)	*P* value
Age (y)	42.8 ± 11.5	47.3 ± 10.1	0.509
Gender (F/M)	58/12	11/1	0.680
WIT (min)	11.2 ± 5.4	10.4 ± 7.2	0.656
CIT (h)	10.1 ± 2.7	9.8 ± 2.5	0.699
Anhepatic time (min)	52.9 ± 11.0	45.6 ± 11.2	0.134
ITBLs appearing after OLT (d)	42.6 ± 14.8	41.8 ± 22.0	0.883

Abbreviations: F, female; M, male; WIT, warm ischemic time; CIT, cold ischemic time.

Procedures for the diagnosis and treatment of ITBLs

In the control group, magnetic resonance cholangiopancreatography (MRCP) were used for the preliminary diagnosis of ITBLs in OLT patients who developed cholangitis, jaundice or liver function abnormality. Medications (including ursodesoxycholic acid, adenosine methionine, prostaglandin-E and antibiotic) were administered as required. Endoscopic retrograde cholangio-pancreatography (ERCP) or percutaneous cholangiodrainage (PTCD) was performed to confirm the ITBLs diagnosis and maintain the patency of the biliary drainage, and partial hepatectomy and/or choledochojejunostomy were performed in some cases if needed. Retransplantation was considered only when the previous strategies failed. All of the previous procedures were kept the same in both groups, except MSCs were injected only in the MSCs group at the time of confirmed diagnosis of ITBLs.

UC-MSCs preparation and transfusions

With the written consent of the parents, fresh human umbilical cords were obtained after birth and collected in phosphate-buffered saline at 4°C after birth. The processing of the umbilical cords and preparation of UC-MSCs were performed at the GMP Stem Cell Laboratory Facility of the biotherapy center in our hospital. Fresh human umbilical cords were cut into 0.5 cm pieces and floated in Dulbecco's modified Eagle's medium containing low glucose, 10% fetal bovine serum, 100 U/mL penicillin and streptomycin at 37°C in a humidified atmosphere with 5% CO_2. The medium was changed every 2 d, and nonadherent cells were removed by washing after 7 d. When well-developed colonies of fibroblast-like cells appeared after 10 d, the cultures were trypsinized and transferred (without dilution) into a new flask for further expansion. When UC-MSCs reached confluency, the cells were detached and characterized using fluorescence-activated cell sorting (FACS) analysis. The specific details of isolation, culture and characterization of UC-MSCs were described in our previous research [28]. For UC-MSC quality control, bacterial, fungal and viral monitoring (including hepatitis B virus [HBV], hepatitis C virus [HCV], human immunodeficiency virus [HIV] and cytomegalovirus) was performed in all umbilical cords and prior to injection. Pathogen-free UC-MSCs were cultured and collected at fourth