

Umbilical Cord Mesenchymal Stem Cell Treatment for Alcoholic Acute-on-chronic Liver Failure: A Case Report and Literature Review

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ABSTRACT

Objective: Alcoholic acute-on-chronic liver failure (ACLF) is a unique clinical entity that is characterized by acute onset, poor prognosis and high short-term mortality. There is no specific and effective treatment for this disease, except liver transplantation.

Methods: Recent scientific development suggests that umbilical cord-derived mesenchymal stem cells (UC-MSCs) might offer an alternative for liver transplantation in selected cases. Herein, we report a case of a 41-year-old man with alcoholic ACLF admitted to our department with jaundice, ascites and lower limbs edema. The patient was treated with anti-infection remedies, plasmapheresis and artificial liver treatment, which only provided transient relief. In the absence of other available therapeutic approaches, we administered three intravenous transfusions of a total dose of 8.5×10^7 UC-MSCs, with the patient's consent.

Results: The patient exhibited decreased jaundice, normalized TBIL level, decreased Child's Pugh scores, and recovered from ACLF.

Conclusion: Therefore, UC-MSCs transplantation played a transitional role in this patient.

Keywords: Alcoholic acute-on-chronic liver failure Mesenchymal stem cells, alcoholic acute-on-chronic liver failure, liver diseases

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INTRODUCTION

Alcoholic liver disease (ALD) is one of the most common diagnoses among liver diseases in Europe and the United States.(1) In China, ALD is the most increasing liver diseases in recent years.(2) Some patients with chronic ALD may rapidly progress towards acute-on-chronic liver failure (ACLF), which is a severe, life-threatening condition, for which liver transplantation is the standard treatment.(3) However, there is a prevailing contradiction between the urgent need for liver transplants and a severe shortage of donor livers, which highlights the necessity to develop new therapeutic strategies for these conditions.

Mesenchymal stem cells (MSCs) are multipotent with self-renewal abilities and the potential to differentiate into various types of cells, including hepatocytes.(4) MSCs have been investigated and clinically used in numerous immune-mediated or inflammatory diseases such as acute GVHD.(5) MSCs have also been used in clinical trials for treatment of liver diseases. Infusions of autologous Bone marrow (BM)-MSCs or umbilical cord (UC)-MSCs have significantly improved liver function in patients with liver cirrhosis (6-8) and liver failure.(9, 10) To explore alternative approaches for treatment of liver diseases, we tried MSC therapies in selected patients. Herein, we report the case of an alcoholic ACLF patient who recovered due to MSC treatment, as indicated by decreased jaundice, normalized TBIL level and decreased Child's Pugh scores. A literature review on MSC treatment of liver diseases was also conducted.

SUBJECTS AND METHODS

Umbilical-cord-derived MSC preparation, culture and identification

The umbilical cords (UCs) were dissected after thorough washing, and the blood vessels were removed. The use of UCs was approved by the Ethics Committee of Qilu hospital of

Shandong University, and written informed consent from the donors was provided by the department of obstetrics at Qilu hospital of Shandong university. The remaining tissues were cut into small pieces (1-2 mm³) and cultured in plates with L-DMEM, supplemented with 10% FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37 °C with 5% CO₂. After 7-12 days, the tissue pieces were removed and the adherent fibroblast-like cells were cultured to confluence. The cells were then passaged at 1×10⁴ cells/cm² in the medium and used after five or more passages.

Fifth- to seventh-passage cells were collected and treated with 0.25% trypsin. Subsequently, the cells were stained with either fluorescein-isothiocyanate-conjugated or phycoerythrin-conjugated monoclonal antibodies in 100 µL of phosphate buffer for 15 min at room temperature, as suggested by the manufacturer. The antibodies used were against human antigens CD29, CD31, CD34, CD45, CD44, CD73, CD90, CD105, and CD271 (BD Pharmingen, USA). The cells were analyzed with a flow cytometer (Guava easyCyte8HT, EMD Millipore, Billerica, USA), and the data were examined with Guava Incyte (EMD Millipore). Positive cells were counted and compared with the signal of corresponding immunoglobulin isotypes.

Case

A 41-year-old man (Han Chinese worker) was admitted to our hospital with right upper quadrant pain and worsening jaundice. The patient was usually in good health, denied other disease history, or long-term use of drugs, or history of Chinese herbal medicine or health products usage, or toxic exposure. His father was healthy, and mother had high blood pressure. Seven days ago, he suddenly had a fever (37.7 °C) and jaundice, with alanine aminotransferase (ALT) of 53 IU/L, total bilirubin (TBIL) of 413 µmol/L, direct bilirubin (DBIL) of 152 µmol/L, serum albumin of 25.3 g/L, peripheral white blood cell (WBC) count of 13.44×10⁹/L, neutrophils 80.7%, and platelet (PLT) 224×10⁹/L. Anti-infection, reduced

glutathione and magnesium isoglycyrrhizinate were used for treatment but only showed a slight increase in serum ALB concentration.

At admission, he had significant jaundice, with ALT of 50 IU/L, TBIL of 419 $\mu\text{mol/L}$, DBIL of 297 $\mu\text{mol/L}$, serum albumin of 32.9 g/L, pre-ALB of 0.03 g/L, prothrombin activity (PTA) of 63%, WBC count of $13.0 \times 10^9/\text{L}$, and PLT $269 \times 10^9/\text{L}$. Clinical examination revealed severe sclera and skin yellowing, visible liver palm and mild bilateral pitting pedal edema. The abdomen was distended with positive tenderness and rebound tenderness on physical examination. No abdominal mass was palpable. Shifting dullness, and enlarged liver and spleen were present. Examination of other systems were normal. Ultrasonography of the abdomen showed massive ascites. A CT scan of the abdomen without intravenous contrast further delineated that the liver volume was enlarged with heterogeneous density (Figure 1). The patient had a history of drinking more than 150 g ethyl alcohol each day for more than 20 years, and smoking 15 cigarettes each day for more than 15 years. Other chronic liver disease etiologies were found through serum tests and case history including hepatitis B, C, D and E virus, CMV, EBV and HIV infection, drug-induced liver injury, and autoimmune liver diseases. Table 1 summarizes the main liver function tests. Based on these clinical presentations, and the results of laboratory and physical examinations, the patient was diagnosed as alcoholic ACLF.

After admission to our hospital, the patient was treated with liver protection and anti-infection remedies for 14 days but did not respond. He was subsequently given plasma exchange, bilirubin adsorption and artificial liver treatment at 3-day intervals for 45 days. After each plasma exchange or artificial liver treatment, serum total bilirubin levels transiently decreased but quickly rebound to the pre-treatment levels. Following this period, he had repeated occurrence of fever, rash, anemia, electrolyte imbalance, continuous jaundice and serum TBIL $> 450 \mu\text{mol/L}$. Liver transplantation was considered but not performed due

to lack of matched donor liver. Further treatments including venous, hypodermic and intramuscular injected drugs, plasma exchange and artificial liver were refused by the patient's family. After informed consent from the patient and approval by the ethics committee of our hospital, the UC-MSCs treatment was applied.

Mesenchymal stem cells-based treatment scheme

Before MSCs treatment, the patient was treated with glutathione, ornithine aspartate, compound glycyrrhizin, ursodesoxycholic acid for anti-oxidation and protecting liver and gallbladder functions, and eperazone-sulbactam and imipenem for anti-infection. Furthermore, plasma exchange treatment was given ten times. In two months, the patient developed recurrent fever, which was identified as drug-induced fever, although the specific drugs were not identified. Since the patient usually developed fever after intravenous treatment, we stopped all intravenous drugs and started cell therapy, in which the patient only took oral compound amino acid and ursodesoxycholic acid. UC-MSCs at a dose of 2.5×10^7 were intravenously administered, after approval from our hospital's IRB. No side-effects were observed during this treatment. The second and third clonal UC-MSCs were given one week and two weeks after the first infusion at the dose of 3.0×10^7 , respectively.

RESULT

After several passages, the adherent cells from UC could form a monolayer of typical fibroblast and plastic-adherent cells (Fig. 2A). H&E staining showed spindle-shaped cells (Fig. 2B), and flow cytometry results demonstrated that the UC-derived cells shared most of their immunophenotypes with MSCs, including positive stromal markers expression (CD29, CD44, CD73, CD90, and CD105) and negative hematopoietic markers expression (CD34 and CD45), endothelial cell marker CD31, and differentiated activated effector cell marker CD271

(Fig. 2C). These results indicated that the cells were undifferentiated and had stem cell characteristics.

Three weeks after the onset of MSC treatment, the patient's jaundice began to decrease, with normalized TBIL level. Other biochemical examinations also returned to normal during the following 24 weeks (Table 1). The patient finally recovered.

ACLF is a unique clinical entity that is characterized by acute onset, poor prognosis and high short-term mortality. Generally, it occurs as a result of various chronic hepatic injuries and usually displays sequential and overlapping severe pathogenic processes that include severe inflammation, hepatocyte necrosis and fibrosis/cirrhosis.(9, 11) Liver transplantation is the standard treatment for such patients. However, limited numbers of donors, long waiting lists, high cost, and multiple complications (such as rejection, problems associated with the long-term use of immunosuppressants, and perioperative morbidity and mortality) have restricted the use of liver transplantation in many ACLF patients. Therefore, new strategies to delay or prevent progression of liver failure are urgently required.

Stem cell-based therapies are emerging as a novel alternative for the treatment of ACLF.(12) Notably, the administration of MSCs was reported to be well-tolerated, and also exhibited beneficial effects in patients with liver failure by enhancing liver function and reducing Child's Pugh/MELD scores, ascites and mortality in small-sample size clinical trials.(6-10) These trials indicated that MSC transfusions are safe and feasible in clinical use for the treatment of chronic liver cirrhosis and liver failure.(6-10) Our patient did not respond to general treatments including liver protection, anti-infection, plasma exchange and artificial liver, which was mainly indicated by persistently worsening liver functions. However, three doses of UC-MSC transfusions markedly improved his liver functions, and he finally recovered from ACLF. The UC-MSC treatment likely played a pivotal role in the recovery because all other therapeutic measures were stopped when the MSC treatment was initiated.

This is consistent with observations from several clinical trials of HBV-associated ACLF patients demonstrating the efficacy of UC-MSC treatment.(9, 10) Although our patient showed normal liver function test results after 24 weeks, it is premature to conclude the long-term therapeutic effect of UC-MSC in this case. Further follow-up is required. However, our report has confirmed the benefit of UC-MSC transfusion in the treatment of alcoholic ACLF when almost all other approaches are exhausted. Depending on the course of our patient's disease, a liver transplantation may still be required in the long-term. Nevertheless, the UC-MSC transfusion offered at least a transitional arrangement before liver transplantation is conducted. Thus, this case along with other reports (9, 10) indicated that UC-MSC transplantation played a transitional role in alcoholic ACLF. While our patient responded well to stem cell therapy, this study has several limitations. First, this is only a case report with no statistical value. Second, although no significant adverse effects were observed in our patient, this was not a well designed study to examine the safety and efficacy of this therapy. Future larger-scale, randomized controlled trials are needed to examine the safety and efficacy of UC-MSC treatment in alcoholic ACLF patients. We wish to examine the dynamics of the infused cells, histological alterations in the liver, optimal administration route, timing and dosage, and the mechanisms underlying the bidirectional interactions between infused MSCs and hepatic inflammatory/fibrotic microenvironments. If these challenges can be resolved, the clinical application of MSCs with controllable and feasible standards will be further warranted.(13)

CONCLUSION

In conclusion, our case report indicated that UC-MSC transplantation via a peripheral vein was well tolerated and at least played a transitional role in a patient with alcoholic ACLF. Its safety, clinical value, pharmacokinetics, pharmacodynamics and mechanisms underlying the

therapeutic effect need further investigation.

AUTHORS' NOTE

Jing-Bo Wang and Ge-Feng Dong carried out the studies, participated in collecting data, and drafted the manuscript. Da-Ying Geng and Xian-Zhong Lu performed the statistical analysis and participated in its design. Zhuang-Bo Mou helped to draft the manuscript. All authors read and approved the final manuscript.

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Table 1: Biochemical parameters of the patient during the treatment period

	ALT (IU/L)	TBIL ($\mu\text{mol/L}$)	DBIL ($\mu\text{mol/L}$)	ALB (g/L)	PALB (g/L)	CHE (U/L)	PT (second)	PTA (%)	WBC ($10^9/\text{L}$)	PLT ($10^9/\text{L}$)	Cr ($\mu\text{mol/L}$)
2011.12.08 (Admission to hospital)	50	418.7	297.1	32.9	0.03	120	16.8	63	13	269	44
2012.01.05	75	523.9	312.8	37.1	0.08	298	20.5	45	15	248	69
2012.02.04	23	569.3	341.3	35.9	0.02	355	20.0	47	8.6	158	63
2012.02.21 (first UC- MSC transfusion)	59	486.5	282.5	35	0.01	212	19.3	50	5.1	199	46
2012.02.28	29	488.1	265.6	34	0.01	167	19.5	49	7.2	186	67
2012.03.16	38	340.1	205.4	33.9	0.02	154	18.7	57	5.6	204	61

Use of Umbilical Cord Mesenchymal Stem Cell in ACLF

2012.03.22	29	244.7	145.4	32.4	0.03	121	20.6	45	5.9	208	55
2012.04.06	32	139.3	78.6	34.2	0.05	145	18.9	52	3.7	165	48
2012.05.11	50	83.7	38.4	42.8	0.11	233	18	56	2.5	110	64
2012.06.5	49	75.6	26.8	46.4	0.13	271	17.1	61	2.6	98	63
2012.06.28	48	68.7	24.3	44.3	0.13	281	17.2	60	3.9	90	62
2013.02.28	46	80.9	31.4	44.7	0.10	289	15.5	70	4.66	105	70

ALT, alanine aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; ALB, albumin; PALB, pro-albumin; CHE, cholinesterase; PT, prothrombin time; PTA, prothrombin activity; WBC, white blood cell; PLT, platelet; Cr, creatinine.

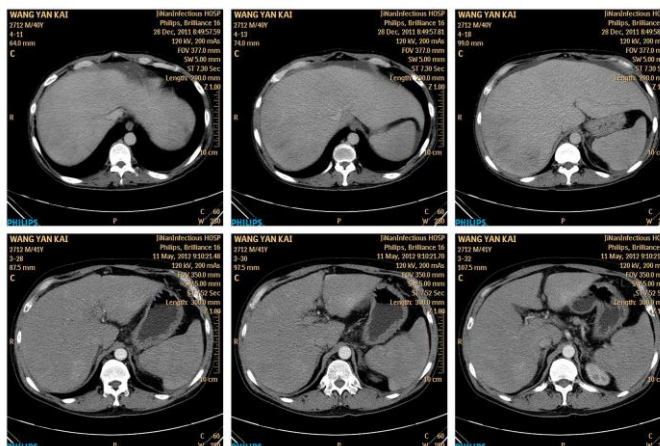


Fig. 1: CT scan before treatment (2011) and one year after treatment (2012).

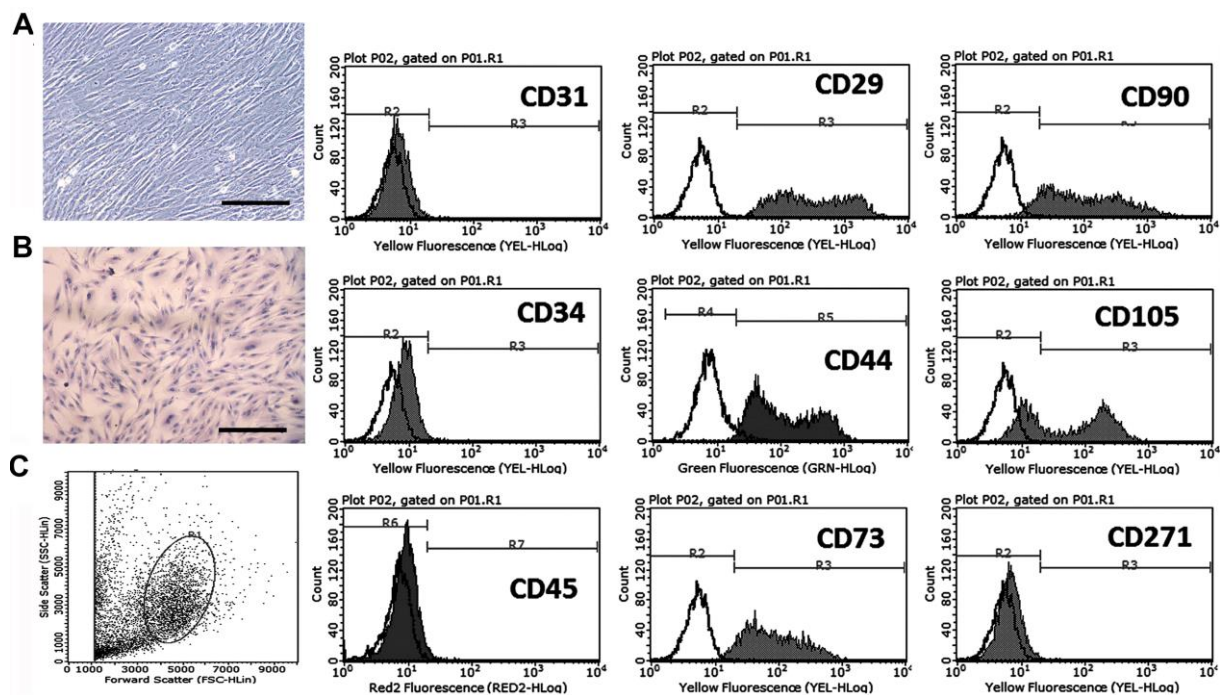


Fig. 2: Characterization of UC-derived MSCs

A. Bright-field image. B. H&E staining image. C. Immunophenotype of MSCs.

The cells were harvested at passage 5, labeled with antibodies specific for the indicated human surface antigens or negative controls, and analyzed by flow cytometry. Scale bars represent 200 μ m.