Autologous Bone Marrow Mesenchymal Stem Cell Transplantation in Liver Failure Patients Caused by Hepatitis B: Short-Term and Long-Term Outcomes

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Our study aimed to investigate the short-term efficacy and long-term prognosis of liver failure patients caused by hepatitis B after a single transplantation with autologous marrow mesenchymal stem cells (MMSCs). A total of 527 inpatients with liver failure caused by hepatitis B were recruited and received the same medical treatments, among whom 53 patients underwent a single transplantation with autologous MMSCs. A total of 105 patients matched for age, sex, and biochemical indexes, including alanine aminotransferase (ALT), albumin, total bilirubin (TBIL), prothrombin time (PT), and Model for End-Stage Liver Disease (MELD), comprised the control group. A total of 120 mL of bone marrow was obtained from each patient and then diluted and separated. Then, the MMSC suspension was slowly transfused into the liver through the proper hepatic artery. The success rate of transplantation was 100%, without serious side effects or complications. Levels of ALB, TBIL, and PT and MELD score of patients in the transplantation group were markedly improved from 2-3 weeks after transplantation, compared with those in the control group. At 192 weeks of follow-up, there were no dramatic differences in incidence of hepatocellular carcinoma (HCC) or mortality between the two groups. Additionally, there were no significant differences in the incidence of HCC or mortality between patients with and without cirrhosis in the transplantation group. *Conclusion:* Autologous MMSC transplantation is safe for liver failure patients caused by chronic hepatitis B. Short-term efficacy was favorable, but long-term outcomes were not markedly improved. In respect to several parameters, this method is preferable for patients with liver cirrhosis and may have potential for reducing their incidence of HCC and mortality. (HEPATOLOGY 2011;54:820-828)

Hepatitis B is a major global health problem and the most serious type of viral hepatitis. It is one of the primary causes of liver cirrhosis, liver failure and is one of the major risk factors of hepatocellular carcinoma (HCC), with approximately 350 million people infected worldwide.[1] The disease is particularly serious in some developing countries, and new, effective therapies are essential, in addition to current medical, antiviral, and immunomodulation treatments and liver transplantation.

Abbreviations: ALT, alanine aminotransferase; CT, computed tomography; ECM, extracellular matrix; EDTA, ethylenediaminetetraacetic acid; FITC, fluorescein isothiocyanate; FBS, fetal bovine serum; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HIV, human immunodeficiency virus; IgG1, immunoglobulin G1; L-DMEM, Dulbecco's modified Eagle's medium with low glucose; MELD, Model for End-Stage Liver Disease; MMSCs marrow, mesenchymal stem cells; MR, magnetic resonance; MSCs, mesenchymal stem cells; PBS, phosphate-buffered saline; PE, phycoerythrin; PT, prothrombin time; TBIL, total bilirubin

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In China, the hepatitis B surface antigen (HBsAg) seropositive rate is 9.09% for individuals over 3 years of age,[2] and hepatitis B promotes social health problems as a result of its potential for serious complications, including liver cirrhosis and liver failure.

Marrow mesenchymal stem cells (MMSCs), one type of somatic stem cells, are characterized by the property of self-renewal and multipotentiality,[3-7] and MMSCs have the following advantages for therapeutic application: ease for isolation and cultivation, high expansion potential, a stable phenotype, substantial immune compatibility, and mild side effects after transplantation. MMSCs have been demonstrated to play an important role in cellular therapy and tissue engineering[8-10] and have significant potential for the treatment of hepatitis B.

In autologous transplantation, MMSCs are derived from the patients themselves, which avoids the potential for immune rejection. At present, autologous MMSCs have been widely applied in the treatment of liver diseases.[11-14] However, some studies on the treatment of chronic liver disease with MMSC transplantation have some limitations, such as a small sample size, lack of controls, and absence of tracing the transplanted cells, short-term observation, and absence of evaluation on long-term efficacy, prognosis, and safety.[15,16] In 2005, we conducted autologous transplantation with MMSCs in liver failure patients caused by hepatitis B,[17] with the longest follow-up reaching 192 weeks. We report our findings, including liver function at 1∼48 weeks after transplantation, symptoms and survival rate, and incidence of HCC during the 192-week follow-up. Patients matched for age, sex, and disease condition were recruited as controls. We aimed to investigate the short-term efficacy and long-term prognosis of liver failure patients caused by hepatitis B after a single transplantation of autologous MMSCs.

Patients and Methods

Study Population. A total of 527 patients with chronic hepatitis B–induced liver failure were recruited from May 2005 to June 2009 from our department. The diagnoses of chronic hepatitis B and liver failure were based on previously described criteria.[18,19] Informed consent was obtained before the study. Studies began after 1 week of the same medical treatments (i.e., reduced glutathione, glycyrrhizin, ademetionine, polyene phosphatidylcholine, alprostadil, and human serum albumin) were performed on all patients. This time point was used as a baseline. All patients were informed about the process of autologous transplantation of MMSCs and volunteered to receive this treatment. A total of 53 patients (group A) accepted the protocol, and transplantations were performed within 3 days after 1 week of the medical treatments mentioned above. In addition, the medical treatments mentioned above were continued throughout the study for all patients. A total of 105 patients (group B) matched for age, sex, and some biochemical indexes, including alanine aminotransferase (ALT), albumin (ALB), total bilirubin (TBIL), prothrombin time (PT), and Model for End-Stage Liver Disease (MELD), served as controls (Table 1). In addition, the patients in each group were divided into subgroups of patients with or without cirrhosis: 39 and 77 patients with cirrhosis in subgroups A1 and B1 and 14 and 28 without cirrhosis in subgroups A2 and B2 (no significantly differences were found). Cirrhosis was diagnosed by the evidence of a small, nodular liver, as shown by ultrasound, computerized tomography (CT), and magnetic resonance (MR), with the exclusion of primary biliary cirrhosis and cirrhosis caused by schistosome.

Table 1. Clinical and Biochemical Features of Two Groups at Baseline

	Transplantation Group (Group A)	Control Group (Group B)	*P* Value
Age, years	42.19 ± 10.80	42.22 ± 11.37	0.988
Sex (male) (%)	50 (94.34)	99 (94.29)	0.989
Liver cirrhosis patients (%)	39 (73.58)	77 (73.33)	0.973
ALT (3 ∼ 35 U/L)	97.51 ± 56.60	98.26 ± 56.73	0.974
Albumin (36 ∼ 51 g/L)	29.21 ± 3.12	29.84 ± 3.86	0.444
TBIL (2 ∼ 23.9 μmol/L)	198.42 ± 104.98	194.92 ± 67.45	0.588
PT (11 ∼ 14.5 seconds)	23.95 ± 4.05	23.49 ± 4.27	0.376
MELD score	30.01 ± 3.99	29.15 ± 3.72	0.126

Data are expressed as the mean ± standard deviation or n (%).

Inclusion and Exclusion Criteria. Inclusion criteria consisted of 15-75 years of age, agreement to informed consent, and a diagnosis of hepatitis B–induced liver failure.[18,19] Exclusion criteria included pregnant and lactating women, antiviral or immunomodulatory therapy within 6 months before surgery, presence of other factors causing active liver diseases (e.g., autoimmune diseases, drug-induced liver disease, alcoholic liver disease, inherited metabolic liver diseases, etc.), concomitant human immunodeficiency virus (HIV) infection or congenital immune deficiency diseases, proven liver cancer or other malignancies, severe diabetes, autoimmune diseases, other important organ dysfunctions (e.g., kidney dysfunction), concomitant infection (e.g., fever, leukocytosis or neutrophilia, and manifestations of abdominal, biliary tract, or lung infection) or other

serous complications (e.g., hepatic encephalopathy, gastrointestinal bleeding, etc.), intolerance to the medical treatments mentioned above, and patients having received, or who would receive, bioartificial liver support therapy or liver transplantation.

Clinical Trial. Our study is registered at ClinicalTrials.gov (NCT00956891), with the registered name of "Long-Term Follow-up of Liver Failure Patients Who Received Autologous Mesenchymal Stem Cells (MSCs) Transplantation." The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the appropriate institutional review committee.

Preparation of MMSCs from Patients with Liver Failure. After skin preparation, sterilization, and local anesthesia, marrow aspiration was performed in bilateral anterior-superior iliac crests. A total of 100-120 mL of human bone marrow was obtained and anticoagulated with 1000 U/mL of Liquaemin (WanBang Ltd., JiangSu, China.) Density-gradient centrifugation was conducted in a laminar air-flow hood; bone marrow was diluted with normal saline and gently added to Percoll separating medium (Sigma-Aldrich, St. Louis, MO) of equal volume, followed by centrifugation at 2500 rpm for 30 minutes. Interphase-containing cells were obtained and washed three times with 10 mL of normal saline. The cell suspension was collected and preserved in 10 mL of normal saline, with 0.2 mL used to seed Dulbecco's modified Eagle's medium with low glucose (L-DMEM) (Gibco BRL, Grand Island, NY) culture medium supplemented with 10% fetal bovine serum (FBS) (Gibco). Cell morphology and growth were then observed. Contamination was avoided. The average number of mononuclear cells isolated from 100-120 mL of bone marrow was $3.4 \pm 3.8 \times 10^8$ or E8.

Autologous MMSC Culture and Identification by Flow Cytometry Analysis. A total of 0.2 mL of cell suspension was incubated at 37°C in a 25-cm^2 culture flask. The culture medium was changed after 3 days and every 2 days thereafter. MMSCs were digested with 0.25% trypsin and 0.1% ethylenediamine tetraacetic acid (EDTA) and passaged (1:2) when 70%-80% cell fusion had occurred. The third passage of MMSCs was digested, rinsed with phosphate-buffered saline (PBS), and grown at a density of 1.0×10^6 cells/mL. Cells were incubated with fluorescein isothiocyanate (FITC)-CD44, PerCP-CD45, and phycoerythrin (PE)-CD34 antibodies (BD Biosciences, Franklin Lakes, NJ) and detected via flow cytometry (FACScan; BD Biosciences), using mouse isotype immunoglobulin G1 (IgG1) as the control. Amplifier mode was linearity mode, flow rate was low, signals and threshold were set, and the gate was set at the target cells.

Autologous MMSC Transplantation. Interventional procedures were performed in an operating room. An electrocardio monitor was used, and the pipe was located at the proper hepatic artery through the arteria cruralis, abdominal aorta, celiac axis, and arteria hepatica communis after local anesthesia. The cell suspension (in 10 mL of normal saline) was slowly transfused into the liver over 20-30 minutes.

Observation and Follow-Up. Observation and follow-up were performed every week for weeks 1-4 and every 12 weeks for weeks 4-192. All patients could choose to have examinations and follow-up at our hospital or at local medical institutions, and communication via telephone was the only method to acquire some patients' information. Some patients were lost during the 192-week follow-up. The success rate of transplantation, side effects, and complications were observed and recorded.

In regards to short-term therapeutic effects, average hospital stay of the two groups (A and B) was 29.27 ± 31.34 and 30.68 ± 35.29 days, respectively. As such, 1-4 weeks were chosen as the evaluation time for short-term therapeutic effects. Self-reported symptoms, such as reduced appetite, abdominal distention, and fatigue, were recorded and compared over the 1-4 weeks after transplantation. ALT, ALB, and TBIL levels and PT and MELD scores were compared from 1 to 4 weeks after transplantation in all patients.

In regards to the long-term therapeutic effects and prognosis, ALT, ALB, and TBIL levels and PT and MELD scores were compared up to 48 weeks after transplantation. At 48 weeks after transplantation, only 6 and 26 patients in groups A and B returned to our hospital for follow-up, and their liver function indices were recorded. Only 15 of the 26 patients in group B had matched baseline indices with the six patients in group A, and their liver functions indices were thus compared up to 48 weeks after transplantation. To evaluate long-term prognosis, the incidence of HCC and survival rates were recorded every 12 weeks after transplantation.

Statistical Analysis. Data of clinical and biochemical features were expressed as mean ± standard deviation and compared using the chi-square and *t* tests. Analysis of long-term turnover were studied by survival analysis, from which the product-limit estimate was used to calculate the rates (i.e., HCC incidence and mortality), and the Kaplan-Meier curve was delineated. All data were analyzed by SPSS 13.0 software (SPSS Inc., Chicago, IL) and a value of $P < 0.05$ was considered statistically significant.

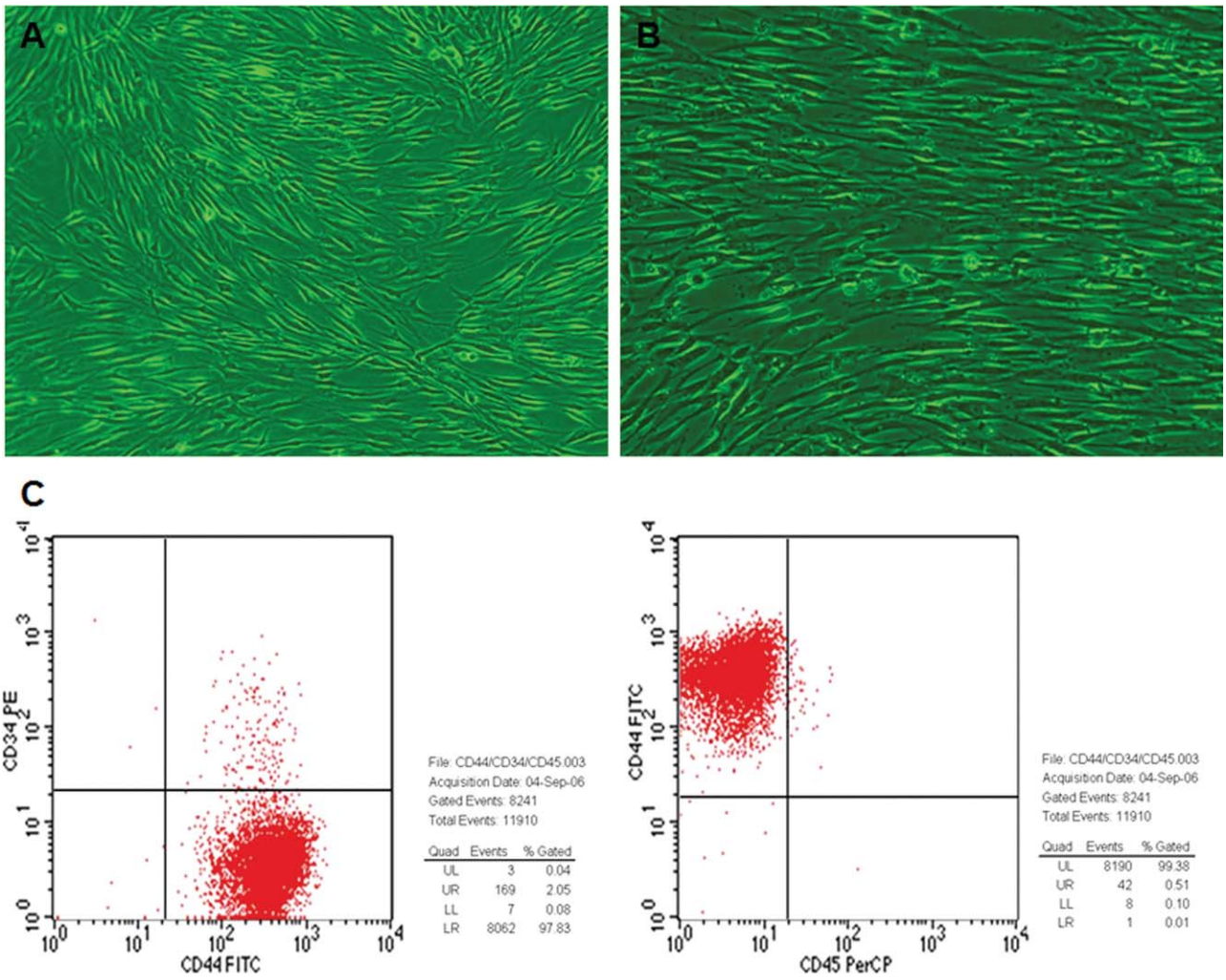

Fig. 1. Morphology and expression of surface molecules CD34, CD44, CD45 of MMSCs from liver failure patients caused by hepatitis B. (A) 100×, origin passage. (B) 100×, third passage. (C) $CD34^{-}CD44^{+}$ and $CD44^{+}CD45^{-}$ cells accounted for 97.83% and 99.38% separately.

Results

Cell Verification. All MMSCs demonstrated a fusiform shape with a high karyoplasmic ratio and were integrated into stable colonies, such as collagenoblasts (Fig. 1A,B). Flow cytometry analysis showed that MMSCs (third passage) from patients with liver failure caused by hepatitis B were positive for CD44 and negative for CD34 and CD45, which was consistent with that of healthy adults (Fig. 1C).

Success Rate and Intra- and Postoperative Side Effects. The collection, separation, and transfusion of MMSCs were successful in all 53 patients, with a success rate of 100%. No serious side effects or complications (including hemorrhage, fever, infection, hepatalgia, etc.) were observed after transplantation.

Short-term Therapeutic Effects. Four weeks after transplantation, patients had improved self-reported symptoms, compared with controls, but this difference was not significant. In the two groups, there were 35 and 68 patients who experienced increased appetite ($P = 0.874$), 33 and 59 patients experienced abdominal distension improvements ($P = 0.465$), and 35 and 61 patients experienced fatigue improvements ($P = 0.334$), respectively.

Liver function comparisons at 1-4 weeks after transplantation indicated that there were no marked differences in ALT levels between the two groups (Table 2). Furthermore, in both groups, there were no dramatic differences in ALT levels between the cirrhosis and noncirrhosis subgroups (Table 3). ALB and TBIL levels of patients in group A were significantly superior to those in group B at week 2 after transplantation (Table 2; Fig. 2A,B). Furthermore, in both groups, there were no significant differences in ALB and TBIL levels between the cirrhosis and noncirrhosis subgroups (Table 3). PT and MELD scores of patients in group A were markedly improved, compared with those in

Table 2. Levels of ALT, Albumin, and TBIL, and PT and MELD Score in the Transplantation Group (Group A) and Control Group (Group B) at 1 ∼ 4 Weeks After Transplantation

Liver Function	Baseline		1 Week		2 Weeks		3 Weeks		4 Weeks	
	A	B	A	B	A	B	A	B	A	B
ALT (U/L)	97.51 ± 56.60	98.26 ± 56.73	92.25 ± 40.54$^{(A)}$	96.76 ± 64.95$^{(a)}$	82.68 ± 54.05$^{(B)}$	91.53 ± 59.93$^{(b)}$	63.17 ± 29.34$^{(C)}$	64.85 ± 38.42$^{(c)}$	55.49 ± 25.91$^{(D)}$	56.89± 32.22$^{(d)}$
Albumin (g/L)	29.21 ± 3.12	29.84 ± 3.86	31.34 ± 2.37$^{(E)}$	31.35 ± 3.00$^{(e)}$	33.81 ± 2.41$^{(F)}$	31.99 ± 2.45$^{(f)}$	34 ± 2.35$^{(G)}$	32.27 ± 2.10$^{(g)}$	35.49 ± 1.74$^{(H)}$	33.79 ± 2.74$^{(h)}$
TBIL (μmol/L)	198.42 ± 104.98	194.92 ± 67.45	165.91 ± 105.70$^{(I)}$	169.03 ± 67.63$^{(i)}$	142.87 ± 115.41$^{(J)}$	155.49 ± 85.21$^{(j)}$	118.94 ± 122.51$^{(K)}$	140.70 ± 112.77$^{(k)}$	104.94 ± 141.76$^{(L)}$	125.95 ± 136.30$^{(l)}$
PT (seconds)	23.95 ± 4.05	23.49 ± 4.27	22.07 ± 4.32$^{(M)}$	22.56 ± 4.55$^{(m)}$	20.54 ± 4.97$^{(N)}$	22.20 ± 5.45$^{(n)}$	19.03 ± 4.70$^{(O)}$	21.34 ± 6.16$^{(o)}$	17.85 ± 5.67$^{(P)}$	19.86 ± 6.37$^{(p)}$
MELD	30.01 ± 3.99	29.15 ± 3.72	28.39 ± 4.28$^{(Q)}$	27.38 ± 4.15$^{(q)}$	24.36 ± 4.86$^{(R)}$	25.52 ± 4.94$^{(r)}$	21.72 ± 5.65$^{(S)}$	23.60 ± 5.56$^{(s)}$	19.14 ± 6.30$^{(T)}$	21.09 ± 6.52$^{(t)}$

ALT: A versus a: $P = 0.553$, B versus b: $P = 0.485$, C versus c: $P = 0.873$, D versus d: $P = 0.851$. ALB: E versus e: $P = 0.814$, F versus f: $P = 0.000$, G versus g: $P = 0.000$, H versus h: $P = 0.000$. TBIL: I versus i: $P = 0.088$, J versus j: $P = 0.020$, K versus k: $P = 0.025$, L versus l: $P = 0.046$. PT: M versus m: $P = 0.448$, N versus n: $P = 0.051$, O versus o: $P = 0.023$, P versus p: $P = 0.041$. MELD: Q versus q: $P = 0.200$, R versus r: $P = 0.200$, S versus s: $P = 0.007$,T versus t: $P = 0.011$.

Table 3. Levels of ALT, Albumin, and TBIL, and PT and MELD Score of Patients in Subgroups at 1 ∼ 4 Weeks After Transplantation

	Baseline				1 Week				2 Weeks				3 Weeks				4 Weeks			
	Group A		Group B		Group A		Group B		Group A		Group B		Group A		Group B		Group A		Group B	
Liver Function	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2
ALT (U/L)	95.38 ± 55.68	103.43 ± 60.83	96.29 ± 56.35	103.68 ± 58.45	91.74 ± 41.70	93.64 ± 38.55	94.94 ± 68.89	101.79 ± 53.45	80.90 ± 54.38	87.64 ± 54.84	90.36 ± 62.83	94.75 ± 52.03	63.10 ± 29.50	63.36 ± 29.98	65.19 ± 42.59	63.89 ± 24.03	55.62 ± 25.72	55.14 ± 27.40	57.18 ± 35.41	56.07 ± 21.65
Albumin (g/L)	29.18 ± 3.46	29.29 ± 1.94	29.90 ± 4.02	29.68 ± 3.44	31.31 ± 2.58	31.43 ± 1.74	31.34 ± 3.10	31.39 ± 2.75	33.85 ± 2.64	33.71 ± 1.68	31.96 ± 2.27	32.07 ± 2.21	34.1 ± 2.55	33.71 ± 1.68	32.1 ± 2.22	32.71 ± 1.70	35.46 ± 1.89	35.57 ± 1.28	33.65 ± 2.73	34.18 ± 2.80
TBIL (μmol/L)	197.41 ± 112.92	202.21 ± 82.46	191.88 ± 63.68	203.18 ± 77.43	162.03 ± 109.76	176.71 ± 96.44	165.90 ± 68.64	177.64 ± 65.20	141.15 ± 108.25	147.64 ± 137.81	155.70 ± 82.84	154.89 ± 92.99	115.72 ± 114.08	127.93 ± 147.89	138.81 ± 107.04	145.89 ± 129.23	99.97 ± 133.48	118.79 ± 167.40	123.44 ± 129.89	132.86 ± 154.94
PT (seconds)	23.94 ± 4.02	23.96 ± 4.28	23.58 ± 4.36	23.23 ± 4.06	22.12 ± 4.34	21.95 ± 4.41	22.53 ± 4.57	22.63 ± 4.58	20.62 ± 4.83	20.33 ± 5.52	22.21 ± 5.43	22.18 ± 5.61	19.19 ± 4.57	18.59 ± 5.19	21.24 ± 6.13	21.61 ± 6.35	17.90 ± 5.64	17.7 ± 5.96	19.76 ± 6.17	20.13 ± 7.02
MELD	29.97 ± 4.02	30.12 ± 4.08	28.70 ± 3.32	30.40 ± 4.48	28.14 ± 4.35	29.11 ± 4.15	27.12 ± 3.83	28.09 ± 4.95	24.32 ± 4.78	24.47 ± 5.26	25.08 ± 4.63	26.74 ± 5.61	21.63 ± 5.55	21.98 ± 6.13	23.13 ± 4.98	24.89 ± 6.86	19.32 ± 6.18	18.61 ± 6.84	21.02 ± 6.07	21.27 ± 7.75

ALT: A1 versus A2 subgroup: $P_{baseline} = 0.593$, $P_{1\ week} = 0.770$, $P_{2\ week} = 0.565$, $P_{3\ week} = 0.769$, $P_{4\ week} = 0.716$. B1 versus B2 subgroup: $P_{baseline} = 0.455$, $P_{1\ week} = 0.134$, $P_{2\ week} = 0.346$, $P_{3\ week} = 0.346$, $P_{4\ week} = 0.528$. ALB: A1 versus A2 subgroup: $P_{baseline} = 0.846$, $P_{1\ week} = 0.729$, $P_{2\ week} = 0.959$, $P_{3\ week} = 0.571$, $P_{4\ week} = 0.590$. B1 versus B2 subgroup: $P_{baseline} = 0.887$, $P_{1\ week} = 0.669$, $P_{2\ week} = 0.694$, $P_{3\ week} = 0.154$, $P_{4\ week} = 0.324$. TBIL: A1 versus A2 subgroup: $P_{baseline} = 0.468$, $P_{1\ week} = 0.414$, $P_{2\ week} = 0.960$, $P_{3\ week} = 0.747$, $P_{4\ week} = 0.402$. B1 versus B2 subgroup: $P_{baseline} = 0.377$, $P_{1\ week} = 0.392$, $P_{2\ week} = 0.685$, $P_{3\ week} = 0.980$, $P_{4\ week} = 0.942$. PT: A1 versus A2 subgroup: $P_{baseline} = 0.984$, $P_{1\ week} = 1.000$, $P_{2\ week} = 0.747$, $P_{3\ week} = 0.369$, $P_{4\ week} = 0.984$. B1 versus B2 subgroup: $P_{baseline} = 0.916$, $P_{1\ week} = 0.983$, $P_{2\ week} = 0.928$, $P_{3\ week} = 0.822$, $P_{4\ week} = 0.988$. MELD: A1 versus A2 subgroup: $P_{baseline} = 0.762$, $P_{1\ week} = 0.437$, $P_{2\ week} = 0.928$, $P_{3\ week} = 0.762$, $P_{4\ week} = 0.333$. B1 versus B2 subgroup: $P_{baseline} = 0.053$, $P_{1\ week} = 0.365$, $P_{2\ week} = 0.172$, $P_{3\ week} = 0.339$, $P_{4\ week} = 0.811$.

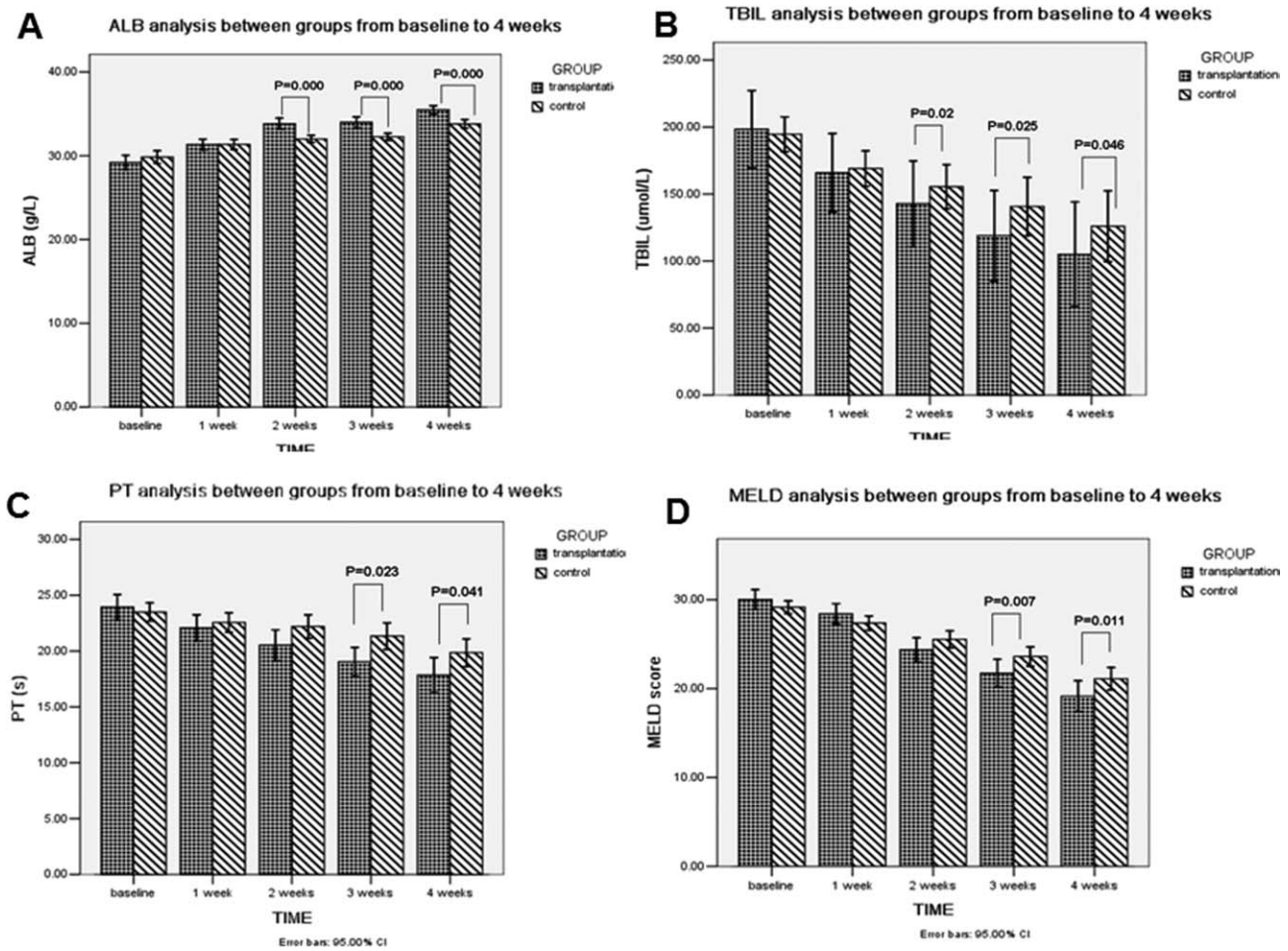

Fig. 2. Liver function comparisons from baseline to 4 weeks after transplantation. (A and B) Albumin (ALB) and TBIL levels of patients in group A were significantly superior to those in group B at week 2 after transplantation. (C and D) PT and MELD scores of patients in group A were markedly improved, compared with those in group B at week 3 after transplantation.

group B, at week 3 after transplantation (Table 2; Fig. 2C,D). Furthermore, in both groups, there were no significant differences in PT or MELD scores between the cirrhosis and noncirrhosis subgroups (Table 3).

Long-term Therapeutic Effects and Prognosis. Liver function comparisons from baseline to 48 weeks after transplantation (Table 4; Fig. 3) indicated that there were no marked differences in ALT levels between the two groups (Table 4(TBL4)). ALB levels of patients in group A were significantly superior to those in group B at 3-24 weeks after transplantation, and significant deviations were not found after 24 weeks (Table 4; Fig. 3A). The improvement in TBIL levels and PT scores of group A was markedly superior to those of group B only at 4-12 week after transplantation (Table 4; Fig. 3B,C). The improvement of MELD scores of group A was markedly superior to that of group B at 3-36 weeks after transplantation (Table 4; Fig. 3D).

In regards to long-term prognosis, only one patient in group A developed HCC at 20 weeks after transplantation, and nine patients in group B developed HCC throughout the 48-week follow-up; there were no significant deviations between these two groups ($P = 0.107$) (Fig. 4A). Furthermore, the survival rate of patients in group A was better than in group B, but significant deviations were not observed from 12 to 192 weeks of follow-up ($P = 0.715$) (Fig. 4B). No HCC was found in the subgroup of patients with cirrhosis from group A, and only one incidence of HCC was observed at 20 weeks after transplantation in the subgroup of patients without cirrhosis from group A; significant deviations were not found. There were no significant deviations between these two subgroups for survival rate ($P = 0.915$) (Fig. 4C).

Discussion

MMSCs demonstrate multipotentiality and can promote liver regeneration, secrete cytokines/growth factors, inhibit inflammation, inhibit activation of liver

Table 4. Levels of ALT, Albumin, and TBIL, and PT and MELD Score of Six Patients in the Transplantation Group (Group A) and 15 Patients in the Control Group (Group B) from Baseline to 48 Weeks After Transplantation

	Baseline		1 Week		2 Weeks		3 Weeks		4 Weeks		12 Weeks		24 Weeks		36 Weeks		48 Weeks	
Liver Function	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
ALT (U/L)	73.17 ± 47.41[(A)]	115.13 ± 64.80[(a)]	58.5 ± 16.53[(B)]	92.47 ± 47.15[(b)]	45.83 ± 14.78[(C)]	67.53 ± 21.64[(c)]	49.17 ± 15.54[(D)]	72.6 ± 43.87[(d)]	36.5 ± 12.42[(E)]	55.2 ± 23.12[(e)]	32.83 ± 9.91[(F)]	42.87 ± 14.68[(f)]	32.5 ± 6.78[(G)]	36.13 ± 10.20[(g)]	34 ± 14.95[(H)]	37.93 ± 9.04[(h)]	37.67 ± 5.16[(I)]	31.93 ± 7.68[(I)]
Albumin (g/L)	29.67 ± 3.14[(A)]	29.4 ± 3.92[(a)]	32.33 ± 3.50[(B)]	31.93 ± 3.26[(b)]	34.17 ± 2.64[(C)]	32.53 ± 2.17[(c)]	35 ± 1.10[(D)]	33.33 ± 1.80[(d)]	35.92 ± 1.11[(E)]	34.47 ± 1.46[(e)]	36.75 ± 2.27[(F)]	33.93 ± 1.98[(f)]	36.93 ± 2.43[(G)]	34.33 ± 2.61[(g)]	37.5 ± 2.31[(H)]	36.17 ± 1.97[(h)]	36.83 ± 2.18[(I)]	36.73 ± 2.71[(I)]
TBIL (μmol/L)	201.17 ± 75.45[(A)]	195.73 ± 56.02[(a)]	160.83 ± 72.14[(B)]	151.4 ± 54.22[(b)]	115.5 ± 54.99[(C)]	131.13 ± 55.42[(c)]	86.67 ± 20.15[(D)]	109.67 ± 53.72[(d)]	66.67 ± 19.10[(E)]	97.8 ± 42.61[(e)]	27.08 ± 6.39[(F)]	42.53 ± 21.17[(f)]	22.17 ± 4.62[(G)]	25.7 ± 10.54[(g)]	27.6 ± 10.29[(H)]	29.33 ± 11.52[(h)]	26.83 ± 5.78[(I)]	23.93 ± 6.89[(I)]
PT (seconds)	26.25 ± 5.34[(A)]	25.95 ± 5.72[(a)]	25.47 ± 5.86[(B)]	24.91 ± 5.11[(b)]	23.68 ± 5.59[(C)]	23.9 ± 5.98[(c)]	21.15 ± 4.11[(D)]	22.89 ± 6.31[(d)]	17.07 ± 4.14[(E)]	22.51 ± 5.66[(e)]	14.82 ± 2.53[(F)]	19.25 ± 3.66[(f)]	16.23 ± 2.56[(G)]	17.53 ± 3.31[(g)]	15.64 ± 3.17[(H)]	17.19 ± 3.07[(h)]	16.32 ± 2.97[(I)]	17.75 ± 3.14[(I)]
MELD	29.58 ± 0.93[(A)]	29.62 ± 3.75[(a)]	27.06 ± 2.03[(B)]	27.72 ± 4.14[(b)]	23.45 ± 1.99[(C)]	25.26 ± 4.01[(c)]	19.79 ± 1.72[(D)]	23.41 ± 4.01[(d)]	17.38 ± 2.08[(E)]	21.36 ± 3.99[(e)]	15.29 ± 2.25[(F)]	19.73 ± 3.49[(f)]	14.67 ± 2.89[(G)]	18.37 ± 2.91[(g)]	15.55 ± 1.73[(H)]	18.79 ± 2.73[(h)]	17.39 ± 2.68[(I)]	18.00 ± 2.52[(I)]

ALT: A versus a: $P = 0.205$, B versus b: $P = 0.112$, C versus c: $P = 0.055$, D versus d: $P = 0.132$, E versus e: $P = 0.066$, F versus f: $P = 0.08$, G versus g: $P = 0.791$, H versus h: $P = 0.622$, I versus i: $P = 0.112$. Albumin: A versus a: $P = 0.910$, B versus b: $P = 0.791$, C versus c: $P = 0.205$, D versus d: $P = 0.045$, E versus e: $P = 0.036$, F versus f: $P = 0.008$, G versus g: $P = 0.018$, H versus h: $P = 0.132$, I versus i: $P = 0.791$. TBIL: A versus a: $P = 0.733$, B versus b: $P = 0.970$, C versus c: $P = 0.470$, D versus d: $P = 0.470$, E versus e: $P = 0.045$, F versus f: $P = 0.036$, G versus g: $P = 0.910$, H versus h: $P = 0.791$, I versus i: $P = 0.424$. PT: A versus a: $P = 0.970$, B versus b: $P = 0.791$, C versus c: $P = 0.970$, D versus d: $P = 0.733$, E versus e: $P = 0.045$, F versus f: $P = 0.018$, G versus g: $P = 0.622$, H versus h: $P = 0.622$, I versus i: $P = 0.302$. MELD: A versus a: $P = 0.791$, B versus b: $P = 0.677$, C versus c: $P = 0.205$, D versus d: $P = 0.036$, E versus e: $P = 0.045$, F versus f: $P = 0.008$, G versus g: $P = 0.045$, H versus h: $P = 0.018$, I versus i: $P = 0.850$.

astrocytes, block the production of extracellular matrix (ECM), and facilitate the degradation of excessive ECM, leading to improvement of chronic hepatitis B, impediment of liver fibrosis, and repair of injured liver tissues.[20] Great progress has been made in the treatment of liver diseases with the use of autologous MMSC transplantation and has included basic research and clinical studies.[11-14,21-24] Yet, there are still a number of problems requiring resolution in clinical practice, including the route of MMSC administration, the number of cells used for transplantation, and homing ability that may affect the efficacy of transplantation.[25-27]

In our previous research, we explored the bionomics of MMSCs from patients with hepatitis B.[22,28,29] Based on these studies, we investigated the safety, short- and long-term therapeutic effects, and prognosis of a single transplantation of autologous MMSCs in patients with liver failure caused by hepatitis B. Results showed that autologous MMSC transplantation was safe for these patients, as no adverse reactions or serious complications (i.e., bleeding, hematoma, infection, etc.) were observed and no elevation in the incidence of HCC was observed over a 192-week follow-up. In respect to short-term efficacy, the improvement in self-reported symptoms was not different between the two groups. In respect to liver function at 1-4 weeks, the improvement in group A was superior to that in group B, as the improvement of ALB and TBIL levels and PT and MELD scores in group A were markedly superior to those in group B at 2-3 weeks after transplantation; however, ALT levels were not markedly changed. In respect to liver function at 1-48 weeks, the observed improvements were not maintained after 36 weeks. Furthermore, during the 192-week follow-up, results revealed no remarkable differences in the incidence of HCC or survival rate between the two groups. These finding implied that autologous MMSC transplantation could not improve the long-term prognosis of patients with liver failure caused by hepatitis B. Furthermore, in group A, no significant difference was observed in the incidence of HCC or survival rate at the different time points between patients with and without cirrhosis. Since cirrhosis is considered one of the most important risk factors for HCC and can lead to a high mortality for patients with hepatitis B,[30,31] our results provided evidence that autologous MMSC transplantation might exert protective effects for cirrhosis patients in regards to the occurrence of HCC and mortality.

Based on the above results, we speculated that autologous MMSC transplantation was safe for patients with liver failure caused by hepatitis B. Autologous MMSC

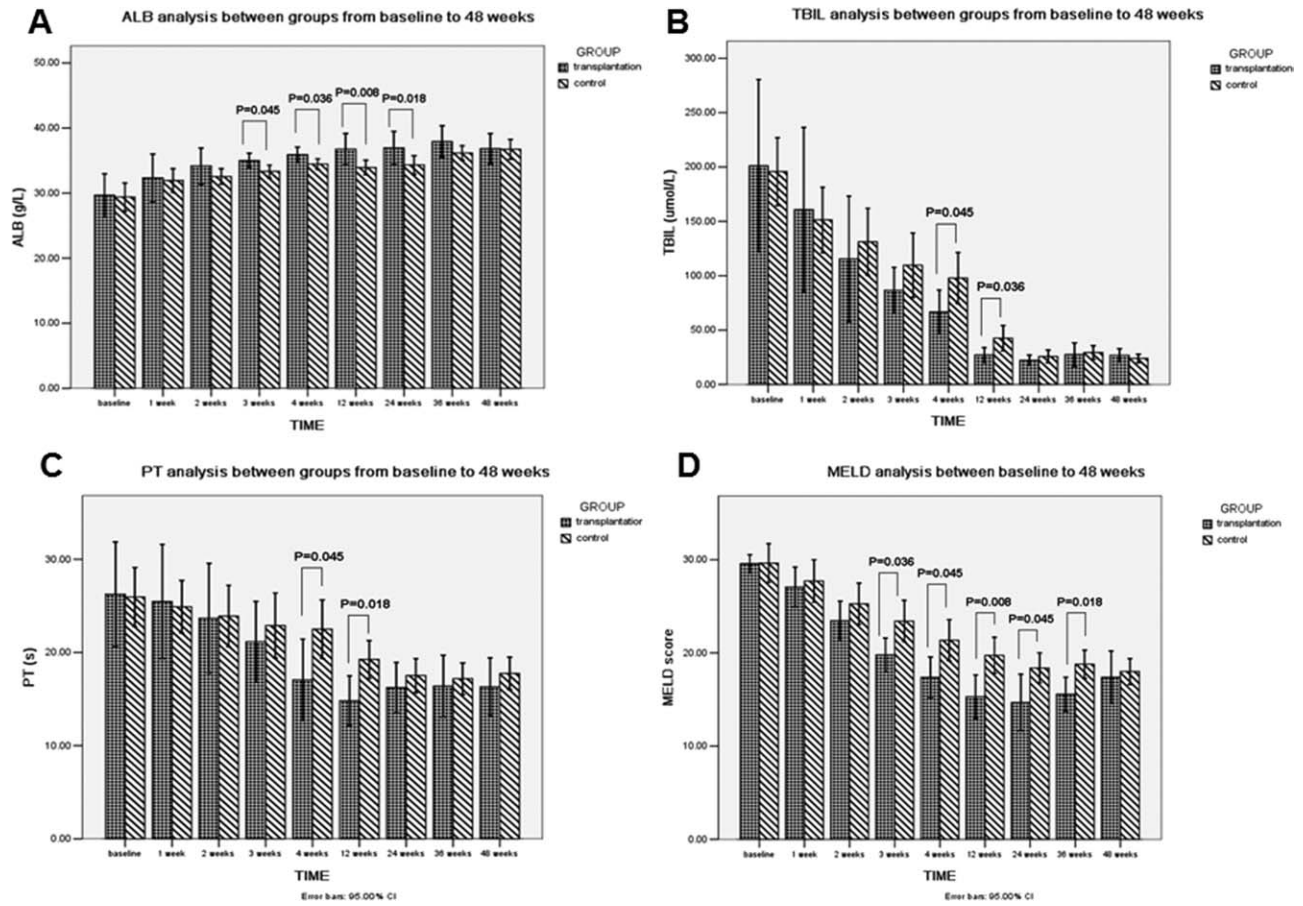

Fig. 3. Liver function comparisons from baseline to 48 weeks after transplantation. (A) Albumin (ALB) levels of patients in group A were significantly superior to those in group B at 3 $\sim$ 24 weeks after transplantation. (B) Improvement of TBIL levels in group A was markedly superior to that in group B only at 4 $\sim$ 12 weeks after transplantation. (C) PT levels of patients in group A were markedly superior to those in group B only at 4 $\sim$ 12 weeks after transplantation. (D) Improvement of MELD levels in group A was markedly superior to that in group B at 3 $\sim$ 36 weeks after transplantation.

transplantation had favorable short-term efficacy (from postoperative weeks 4 to 36) and played important roles in repair after acute liver injury as well as improved disease condition and mortality. Also, for patients with cirrhosis, autologous MMSC transplantation might exert better protective effects in regards to the occurrence of HCC and mortality, but could not markedly improve the long-term prognosis of these patients.

In addition, the transfusion of MMSCs was performed through the proper hepatic artery. However, it

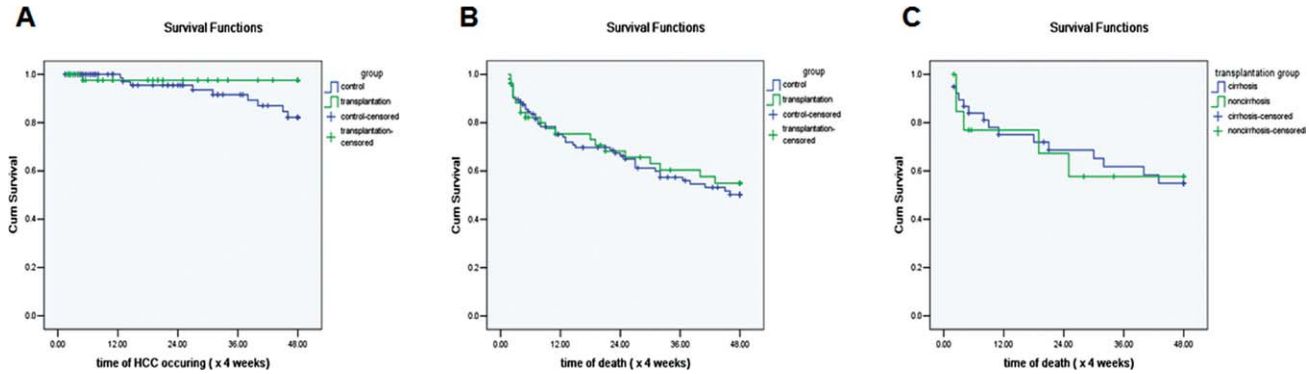

Fig. 4. Long-term prognosis. (A) Only one case in group A developed HCC at 5 months after transplantation and nine cases in group B developed HCC through 12 months in the follow-up ($P = 0.107$). (B) No significant difference was found between these two groups in survival rate ($P = 0.715$). (C) No significant difference was observed in survival rate between 2 subgroups in group A. ($P = 0.915$).

has been shown to be inappropriate to perform the transfusion through the hepatic artery,[26] and transfusion through peripheral veins may achieve more favorable outcomes.[12,14] Furthermore, the limited number of MMSCs in the bone marrow from patients for transfusion[32] and that the homing ability was difficult to increase are the main causes of the compromised efficacy of autologous MMSC transplantation, and this may be why our autologous MMSC transplantation did not achieve acceptable long-term effects on prognosis. *In vitro* proliferation of autologous MMSCs and multiple transplantations with MMSCs with high purity and high density may be the key factors for improving the efficacy of transplantation.

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