



Safety and Efficacy of Autologous Bone Marrow Stem Cell Transplantation Through Hepatic Artery for the Treatment of Chronic Liver Failure: A Preliminary Study

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ABSTRACT

This study was performed to determine the safety and tolerability of injecting autologous bone marrow stem cells (BMC) (CD34+) into four patients with liver insufficiency. The study was based on the hypothesis that the CD34+ cell population in granulocyte colony stimulating factor (G-CSF) mobilized blood and autologous bone marrow contains a subpopulation of cells with the potential for regenerating damaged tissue. We separated the CD34+ stem cell population from the bone marrow. The potential of the BMC to differentiate into hepatocytes and other cell lineages has already been reported. Several reports have also demonstrated the plasticity of hematopoietic stem cells to differentiate into hepatocytes. Recently Sakaida demonstrated reduction in fibrosis in chemically induced liver cirrhosis following BMC transplantation. From a therapeutic point of view, chronic liver cirrhosis is one of the targets for BMC transplantation. In this condition, there is excessive deposition of extracellular matrix and hepatocyte necrosis. Encouraged by this evidence that the CD34+ cell population contains cells with the potential to form hepatocyte-like elements, four patients with liver insufficiency were given G-CSF to mobilize stem cells. CD34+ cells (0.1×10^8) were injected into the hepatic artery. No complications or specific side effects related to the procedure were observed; four patients showed improvements in serum albumin, bilirubin and ALT after one month from the cell infusion.

ALATE STAGE of progressive hepatic fibrosis characterized by distortion of the hepatic architecture and formation of regenerative nodules contributes to cirrhosis. Orthotopic whole organ liver transplantation has significantly improved the prognosis among patients with end-stage or metabolic liver diseases.^{1,2} But it has several limitations such as the shortage of organ donors, high cost (estimated at \$150,000 or more during the first year following transplantation and marks of complications in chronically debilitated often malnourished patients. The incidence of chronic liver failure is expected to increase over the next 10 years as a result of the silent epidemic of hepatitis C. As a result of the shortage of donor organs, potential liver transplant patients must often await a donor liver for years. Only about 5000 patients each year receive a solid liver transplantation. Clearly, the vast majority of patients with liver diseases cannot rely on organ transplantation as a solution. There is an urgent need for new technologies for

acute and long-term support of patients with damaged livers. Case studies by independent investigators have described the administration to humans of human liver cell preparations.

Over the past 30 years, a significant body of scientific literature has shown that in animals infused hepatocytes engrafted into host tissue survive, proliferate, function, and participate in the regenerative process.^{3,4} Transplantation

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Table 1. Biochemical and Fibrotic Markers of Patients Following Autologous Bone Marrow Stem Cell Transplantation

	Patient 1		Patient 2		Patient 3		Patient 4	
	Before Tx	After Tx	Before Tx	After Tx	Before Tx	After Tx	Before Tx	After Tx
ALT (U/L)	126	56	156	55	95	79	105	76
Serum Albumin (g/dL)	3.3	3.8	2.8	3.3	2.9	3	3.8	4.1
Serum bilirubin (mg/dL)	1	0.9	1.2	0.9	1.9	1.4	1.5	1
Hyaluronic acid (ng/dL)	295	248	340	31	380	341	278	232
APRI*	2.5	2.1	2.5	2.2	2.9	2.5	2.4	2.1
Child-Pugh score	9	6	9	6	10	7	8	6
MELD Score	19	11	20	18	21	19	18	18

A.P.R.I., ALT to platelet ratio; MELD, model for end-stage liver disease.

*APRI \leq 0.5 = absence of fibrosis; APRI \leq 1.00 = absence of cirrhosis; APRI \geq 1.5 = presence of fibrosis; APRI \geq 2.00 = presence of cirrhosis.

of hepatocytes into the spleen or liver has been shown to correct inherited defects in metabolism in numerous animal models. These cells almost completely repopulate a host liver under conditions where the host liver cells have a reduced life-span (as in the FAH-deficient mouse model), providing hepatic function during acute liver failure induced by a variety of insults. For example, they have improved liver function and prolonged survival in carbon tetrachloride-induced models of cirrhosis.⁵ Primary hepatocytes remain the choice for transplantation to treat patients with severe acute end-stage chronic and metabolic liver diseases. The scarcity of human donor livers and the absence of proliferation of cultured hepatocytes are major limiting factors for the success of hepatocyte therapy. Preliminary experience with clinical hepatocyte transplantation during the past decade has provided proof of the concept that cell therapy can be effective for treatment of some liver diseases.⁵⁻⁸ Due to the worldwide shortage of donor hepatocytes and perceived inherent risks of infection and rejection associated with xenogenic cells, there is a need to evaluate alternate sources of hepatocytes.⁹ In this connection, embryonic stem cells hold much promise in the future, although constrained by ethical issues. Circulating hematopoietic stem cells contribute to the repair of solid organs, like the liver, offering promise in the future. Stem cells within the adult hematopoietic system could potentially be clinically useful to generate hepatocytes to replace damaged or deficient liver tissue. Peterson et al first reported that rodent bone marrow cells were able to give rise to oval cells and hepatocytes upon transplantation into lethally irradiated rats.^{5,10-13} Wang et al¹⁴ showed that in the presence of hepatic injury, human hematopoietic stem cell and progenitor cell populations had the capacity to generate cells within the recipient liver to synthesize and

secrete human albumin into the sera of mice after transplantation. Recently, an Esch et al reported that portal administration of autologous CD133⁺HSCs accelerated liver regeneration.¹⁵

Recently, reports by Lagasse et al⁹ and by Wager's et al have demonstrated the plasticity of hematopoietic stem cells to differentiate into hepatocytes. Recently, Sakaida et al have demonstrated that bone marrow stem cell transplantation reduced fibrosis in chemically induced liver cirrhosis. From a therapeutic point of view, chronic liver cirrhosis is one of the targets where bone marrow cell transplantation might be employed. In this condition, there is excessive deposition of extracellular matrix and hepatocytes necrosis.¹⁶ Recently, a clinical study by Nagy Habib and his group determined the safety and tolerability of injecting autologous CD34⁺ cells into five patients with liver insufficiency with 2 months follow-up.¹⁷ In the present study, we have reported 6-month follow-up of four cases of established liver cirrhosis who underwent autologous bone marrow stem cell transplantation. We characterized the potential population of bone marrow stem cells that participated in the regeneration process.

METHODS

Selection of Patients

This clinical study was approved by Institutional Ethics Committee. Informed consent was obtained from each patient to carry out the study.

Inclusion Criteria

Inclusion criteria were age 20 to 60 years; chronic liver failure, abnormal serum albumin, bilirubin, aspartate amino transferase (SGOT), prothrombin time, and unsuitable for liver transplantation. Female patients of child-bearing potential used reliable and

Table 2. Clinical Investigations of the Patients Before Transplantation

Patient	Diagnosis/Investigations/Clinical Signs								
	Cirrhosis	Hepatitis	Splenomegaly	Hepatomegaly	Acites	Jaundice	Weight Loss	Fever	Abdominal Pain
Patient 1	Yes	HBV	Yes	Yes	No	No	No	Yes	Yes
Patient 2	Yes	HCV	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Patient 3	Yes	HCV	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Patient 4	Yes	HCV	Yes	Yes	Yes	No	No	Yes	Yes

Table 3. Dosage of Cells, Route of Infusion of Cells and Postinfusion Observations

	Dosage of Cells	Route of Infusion	Postinfusion Observations					
			Bleeding	Pain	Fever	Nausea	Vomiting	Infection
Patient 1	0.1×10^6	Hepatic artery	N	N	N	N	N	N
Patient 2	0.1×10^6	Hepatic artery	N	N	N	N	N	N
Patient 3	0.1×10^6	Hepatic artery	N	N	N	N	N	N
Patient 4	0.1×10^6	Hepatic artery	N	N	N	N	N	N

appropriate contraception. Patients must have the ability to give informed consent.

Exclusion Criteria

Exclusion criteria were patients aged less than 20 or more than 60 years, hepatopulmonary diseases, liver tumors, or history of other cancer. Patients with serum creatinine concentrations >1.5 mg/DL, active infection (including HIV), pregnancy or lactation, recurrent gastrointestinal bleeding, spontaneous bacterial peritonitis, or inability to give informed consent. This clinical study included four subjects with established cirrhosis and portal hypertension or altered liver functions.

Granulocyte-Colony Stimulating Factor Injection

Patients who fulfilled the inclusion criteria were admitted in the Intensive Liver Care Unit. Granulocyte colony-stimulating factor (G-CSF; 300 mcg/mL) was administered subcutaneously for 5 days daily as a single dose.

Bone Marrow Aspiration and Isolation of CD34⁺ Cells Using MACs

A standard bone marrow aspiration procedure was followed, which was approved by the Institution Ethical committee. After 5 days of G-CSF injections, bone marrow was aspirated from iliac crest. This procedure was done by a trained hemato-oncologist/medical oncologist. Briefly, the patient was taken in to the operating theatre and general anesthesia was given. For the iliac crest puncture, the needle was pointed directly at the top of the crest and the bone was pierced downward and slightly outward, in its center. The margins of the crest can be held, the surface of the bone reached a short distance below the skin, and the cavity quickly entered without danger to the patient. Approximately 100 mL of bone marrow was aspirated into a sterile heparin-coated container. After the collection, stem cells were enriched from the bone marrow using standard procedures: the bone marrow was layered over an equal volume of Ficoll-Paque (1.077 g/mL). Mononuclear cells (MNC) were recovered from the gradient interface and washed twice with normal saline (supplemented with 5% dextrose) after centrifugation at 1200 rpm/min for 20 minutes. MNCs were incubated for 45 minutes at 4 °C with the CD34 antibody directly labeled onto microbeads. (MACs, Miltenyi Biotec), washed with MACs buffer, and placed on a column in the miniMACs cell separator (Miltenyi Biotec). The labeled cells, separated using a high gradient magnetic field, were eluted from the column after their removal from the magnet. The viability of the cells was examined using the trypan blue dye exclusion test. The cells were constituted in 10 mL of normal saline.

Flow Cytometric Analysis

Isolated bone marrow cells (1×10^4 per mL) were labeled with primary antibody (CD34) as per the standard protocol. Then

phycoerythrin (PE)-conjugated secondary antibody was labeled with unconjugated primary antibody. Stained cells were analyzed on a FACS Calibur flow cytometer to count the percentage of CD34⁺ cells.

Infusion of Cells to the Patient

The procedure was performed under local anesthesia through femoral arterial access. Initially visceral arterial anatomy was evaluated by angiography. Selective catheterization of the hepatic artery was performed with a 6F guiding catheter. Through the guiding catheter a 3- to 4-mm balloon catheter was negotiated over the guide wire into the hepatic artery. The cells were infused through the distal lumen of the inflated catheter at the rate of 1 mL/min. No procedure-related complications were encountered (Table 3).

Follow-Up Visits

Patients were checked frequently for signs of fever, chills, hives, and angina pectoris while the bone marrow was being infused. At 5 days postinfusion, patients were discharged from the Liver Intensive Care Unit and followed on days 7, 15, 30, 60, 90, 120, 150, and 180.

Laboratory Tests Performed at Each Visit

Liver function parameters, complete blood counts, prothrombin time, and kidney function tests were performed at each visit.

HA Assay

The hyaluronic acid (HA)-ELISA test was performed at each visit to assess serum HA concentrations, which show good prognostic value for the complications of liver cirrhosis. Serum HA levels have also been shown to be related to the degree of fibrosis. High levels of serum HA have been detected in patients with liver cirrhosis of various etiologies. The HA assay is an enzyme-linked binding

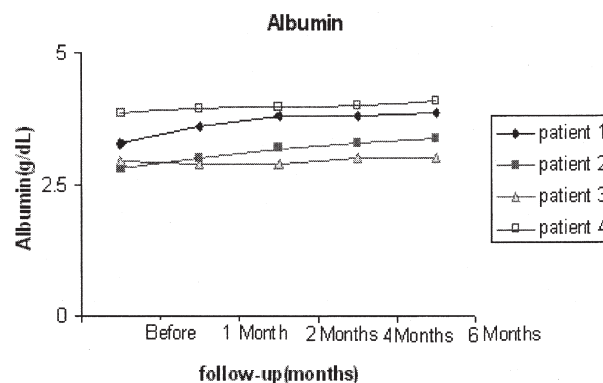


Fig 1. Albumin level before and after cell transplantation.

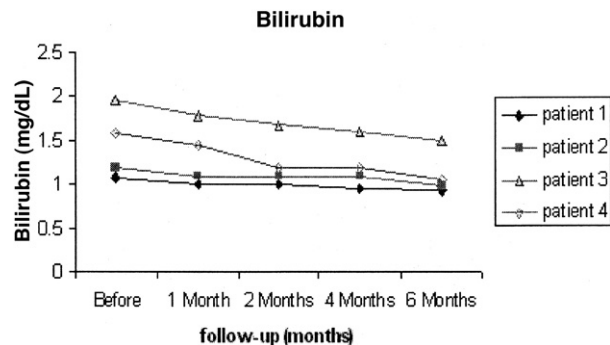


Fig 2. Bilirubin level before and after cell transplantation.

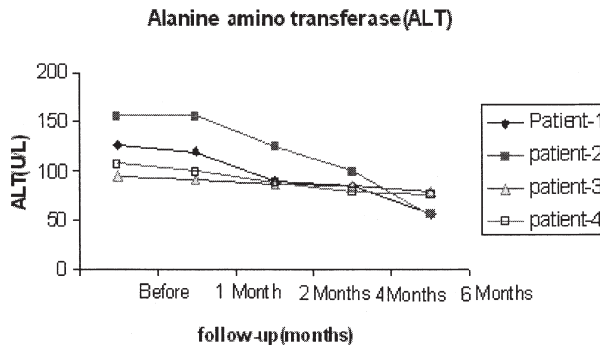


Fig 4. Aspartate amino transferase level before and after cell transplantation.

protein assay that uses hyaluronic acid binding protein (HABP) to capture HA. Diluted samples are incubated in HABP-coated microwells to allow the HA in the sample to bind to the HABP. After a second washing to remove unbound materials, HABP conjugated to horseradish peroxidase is added to form linkages with bound HA. After washing to remove unbound conjugate, chromogenic substrate is added and the intensity measured at 450 nm. The resulting absorbance values are compared to a calibration curve constructed using reference solutions.

RESULTS

Purity and Viability of Bone Marrow Stem Cells

Total bone marrow aspirated from the ilium of the patients was 100 mL with a mean of 0.1×10^8 mononuclear cells (MNCs). The mean purity of bone marrow stem cells was 95.0%; their mean viability was 92.50%.

Clinical Improvement

In all the patients, cells were infused via the hepatic artery. Patients were given 0.1×10^8 cells as a single dose. All procedures were performed without any specific side effects or complications except mild pain at the infusion site. There was no hypersensitive or febrile reaction. Nausea and vomiting were seen following cell infusion. The patients were discharged from the hospital after 5 days of observation. In all patients, there was improvement in appetite. The etiology, diagnosis, clinical signs, results of investigations, cell concentrations, and postinfusion observations for each patient are shown in Tables 1 and 2. Patient 1, patient 2,

and patient 3 completed 6 months follow-up, whereas patient 4 completed 1 year follow-up. Urine creatinine values were normal in all patients.

Patient 1 showed good clinical improvement in all liver function parameters from the second month after cell infusion. The liver function values of this patient after 6 months were albumin: 3.7 gm/dL; SGOT: 41 IU/L; ALP: 74 IU/L; bilirubin: 0.9 mg/dL; and total protein: 7.6 g/dL.

After cell infusion patient 2 showed a gradual increase in serum albumin level to 3.26 gm/dL [2.8 gm/dL before cell infusion], gradual decrease in ALP level to 102 IU/L [235 IU/L before], and gradual increase in total proteins to 6.9 g/dL [5.8 g/dL before]. But no change was observed in SGOT level [56 IU/L to 55.4 IU/L] or bilirubin, 0.87 to 0.8 mg/dL. Patient 3 showed a slow decrease in serum SGOT level to 79 IU/L [95 IU/L before] but only a slight change in ALP to 213 IU/L [208 IU/L before], total proteins as 5.9 g/dL to 6.5 g/dL. There was no change in albumin level 3.0 gm/dL [2.94 gm/dL before]. Patient 4 showed serum albumin levels to be 3.87 gm/dL to 4.1 gm/dL, SGOT as 106 IU/L to 76 IU/L, ALP as 169 IU/L to 156 IU/L, bilirubin as 1.58 mg/dL to 1.06 mg/dL, and total protein, 7.29 g/dL to 7.20 g/dL (Figs 1–6).

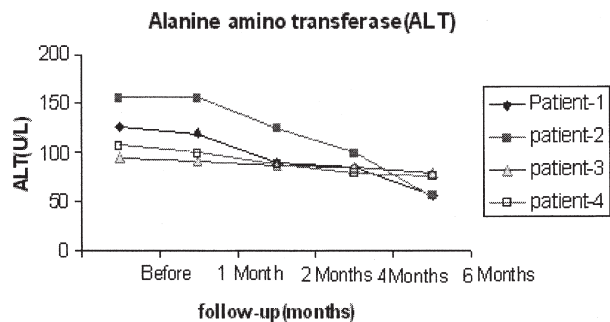


Fig 3. Alanine amino transferase level before and after cell transplantation.

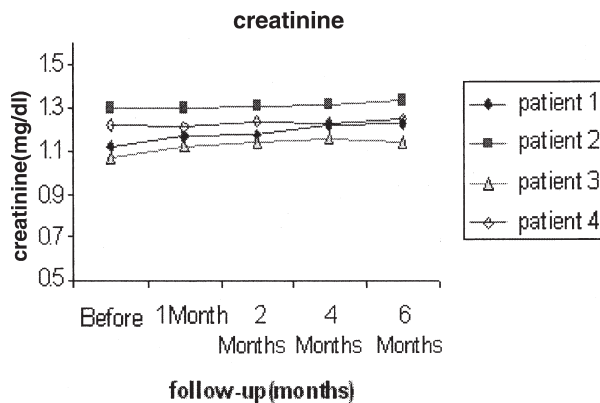


Fig 5. Serum creatinine level before and after cell transplantation.

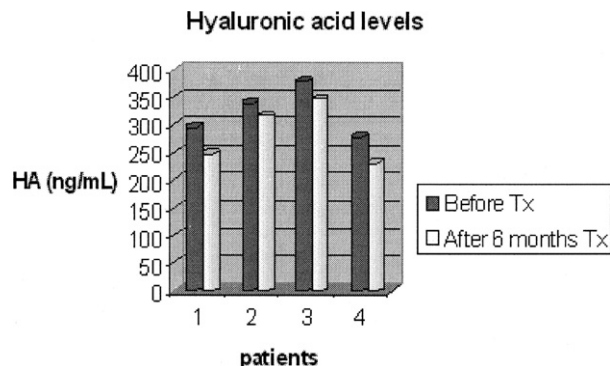


Fig 6. Serum hyaluronic acid level before and after cell transplantation.

DISCUSSION

End-stage liver cirrhosis is an irreversible phenomenon, characterized by loss of hepatocytes and increased extracellular fibrosis. Orthotopic liver transplantation has significantly improved the prognosis among patients with end-stage and metabolic liver diseases.^{1,2} But it has several limitations such as the shortage of organ donors and the high cost. Recently, several experimental studies have shown beneficial effects of bone marrow stem cell rejuvenation of the liver. Differentiation of bone marrow cells into hepatocytes has been shown by Y-chromosome detection upon autopsy analysis of human female recipients of bone marrow cells from a male donor. Lagasse et al reported that purified hematopoietic stem cells could differentiate into hepatocytes. Wagers et al also showed evidence of plasticity in adult hematopoietic stem cells. Several recent studies have explored the potential of bone marrow cells for liver cirrhosis.

In the present study we reported 6 months follow-up of four cirrhosis patients who received autologous bone marrow transplantations through the hepatic artery. Among four patients, one has completed more than 1 year follow-up. In this study, none of the patients developed a significant side effect. At 6 months follow-up, there was no deterioration of any vital organ. Further, there was an increase in albumin among three patients; whereas one patient showed no change. In the three patients who maintained bilirubin levels more than 1.5 mg/dL, there was a fall after 1 month to normal after 2 months posttransplantation. One patient who maintained a bilirubin more than 2.0 mg/dL displayed a value of 1.7 mg/dL after 2 months posttransplantation. Overall, there was a significant improvement in liver function parameters like ALP and SGOT.

In conclusion, this study suggested the safety and efficacy of the autologous bone marrow transplantation through the

hepatic artery. Here we have shown that there was no functional change in kidney function during follow-up. Although Mehdi et al noted kidney failure following transplantation through the hepatic artery. Furthermore, there was no increase in the ascites as was also reported by Mohamadnejad et al.¹⁸

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