

*Forum Minireview***Novel Findings for the Development of Drug Therapy for Various Liver Diseases:****Current State and Future Prospects for Our Liver Regeneration Therapy Using Autologous Bone Marrow Cells for Decompensated Liver Cirrhosis Patients**Taro Takami¹, Shuji Terai^{2,*}, and Isao Sakaida²¹*Division of Laboratory, Yamaguchi University Hospital, ²Department of Gastroenterology & Hepatology, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-kogushi, Ube, Yamaguchi 755-8505, Japan**Received July 6, 2010; Accepted July 20, 2010*

Abstract. We have developed an in vivo mouse model [the green fluorescent protein (GFP) / carbon tetrachloride (CCl₄) model] and reported that infused GFP-positive bone marrow cells administered via a tail vein efficiently repopulated cirrhotic liver tissue under conditions of persistent liver damage induced by CCl₄. Moreover, bone marrow cells infused into the liver improved liver function and ameliorated liver fibrosis with higher expression of matrix metalloproteinase 9 (MMP-9), consistent with improved survival rate. Based on these findings, we started a multicenter clinical trial of autologous bone marrow cell infusion (ABMi) therapy for decompensated liver cirrhosis patients and demonstrated the efficacy of this approach without unexpected complications. However, this therapy involves bone marrow aspiration under general anesthesia and is not indicated for patients for whom general anesthesia is difficult. We therefore aimed to develop a new liver regeneration therapy in which cells having a curative effect on liver cirrhosis are isolated and cultured from a small amount of autologous bone marrow aspirated under local anesthesia and infused back into the same subject. Herein, we present results for the GFP/CCl₄ model and ABMi therapy and future prospects for a new liver regeneration therapy.

Keywords: liver cirrhosis, bone marrow, stem cell, liver regeneration, autologous bone marrow cell infusion (ABMi) therapy, liver disease

1. Introduction

Today, anti-hepatitis virus therapy using interferon for hepatitis C patients is well developed, but interferon is not indicated for patients with decompensated liver cirrhosis. When decompensated liver cirrhosis or other severe liver disease occurs, the only curative therapy is currently liver transplant (liver transplant from either a living or a brain dead donor). However, transplants are not widely performed in Japan, due to various problems including a chronic donor shortage, surgical invasiveness, risk of immunological rejection, and medical costs.

In many cases, symptomatic treatment is the only option. To compensate for this, development of new regenerative therapies for liver cirrhosis is an urgent task.

In 2000, Theise et al. reported the existence of Y chromosome-positive cells in livers with chronic inflammation in autopsy female cases of therapeutic bone marrow transplantations with male donors [transplantation from male (XY) donors to female (XX) patients], and those findings suggested the existence of pluripotent stem cells among bone marrow cells (1, 2). Since then, attention has been focused on bone marrow (stem) cells as a cell source for a liver regenerative therapy worldwide (3).

We have established a mouse model to evaluate differentiation from bone marrow cells to liver cells [the green fluorescent protein (GFP) / carbon tetrachloride

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Published online in J-STAGE on February 22, 2011 (in advance)
doi: 10.1254/jphs.10R13FM

(CCl₄) model] and subsequent proliferation and have reported that in the specific environment of chronic inflammation, bone marrow cells differentiate to albumin-positive cells with a certain efficiency. In that process, liver functions, liver fibrosis, and survival rate all show significant improvements (4–6). Therefore, a clinical study [Autologous Bone Marrow Cell Infusion (ABMi) Therapy] was started in November 2003 based on the results of these basic studies (7). In addition, in 2005, a multicenter clinical trial, a liver regeneration with cell transplantation (LRCT) study, was started. As of the time of this writing, other clinical trials for a liver regeneration are also being conducted in Korea, India, Brazil, Germany, and Iran (3). During this same period, new cell therapies have been developed, including cell therapy using CD34-positive cells induced with granulocyte-colony stimulating factor (G-CSF) in the UK (8, 9) and a therapy with portal administration of CD133-positive mononuclear cells in Germany (10, 11). This article provides an overview of the development of ABMi therapy for liver cirrhosis to date, and the future outlook.

2. Basic study: in vivo mouse model of monitoring of bone marrow cells to liver cells and subsequent proliferation (the GFP/CCl₄ model)

We have conducted basic studies using a model of persistent liver damage induced by CCl₄. The GFP/CCl₄

model that we developed and reported has the following characteristics (4, 5) (Fig. 1): 1) an environment of chronic liver damage induced by repeated administration of CCl₄ (2 times/week); 2) the inflammatory condition is also maintained by repeated administration of CCl₄ after bone marrow cell transinfusion; and 3) assuming transinfused autologous bone marrow cells, the donor is a GFP-transgenic mouse that is isogenic with the recipient.

In this model, repeated peritoneal administration of CCl₄ (0.5 ml/kg body) was performed over 4 weeks (8 times) in 6-week-old female C57BL/6 mice to prepare the chronic liver injury and fibrosis (liver cirrhosis) model in mice used as recipients. Cells in whole bone marrow isolated from the femurs of isogenic male GFP transgenic mice were then washed and injected into the recipient mice via a tail vein (peripheral vein). Administration of CCl₄ was continued after this, and the effect in improving liver function was evaluated over time. Improvement in serum albumin levels (4), a significant increase in survival rate, and decreased hepatic fibrosis assessed by Sirius-red staining were seen following administration of bone marrow cells (6). Bone marrow-derived GFP-positive cells were confirmed to produce collagenases, including matrix metalloproteinase (MMP)-2 and -9, during these processes (6). Based on the above basic study, administration of autologous bone marrow cells via a peripheral vein in a chronic liver injury environment is thought to improve liver functions and reduce liver fibrosis and to significantly improve

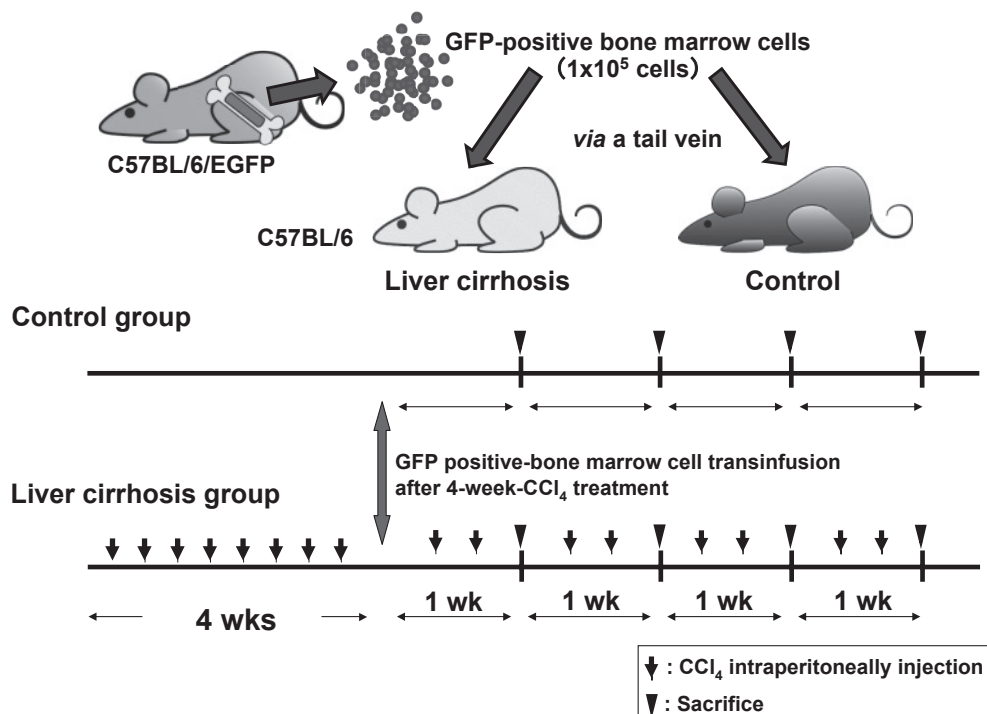


Fig. 1. The mouse GFP/CCl₄ model. The 6-week-old female C57BL/6 mice were treated with CCl₄ twice a week for 4 weeks to induce persistent liver damage. Then 1×10^5 bone marrow mononuclear cells from GFP transgenic C57BL/6 male mice were transinfused through a tail vein. After this transinfusion, CCl₄ treatment twice a week was continued.

vital prognosis in recipients. A later investigation showed that fibroblast growth factor (FGF) has an important function as a factor contributing to this process (12). During early stages of autologous bone marrow cell transfusion, homeobox-containing genes and helix-loop-helix (HLH) transcriptional regulatory genes were also confirmed to be induced by self-organizing-map-based gene expression (13), and apolipoprotein A1 (apoA1) was induced in serum by proteomics analysis (14), suggesting useful bio-markers for liver regeneration.

3. Clinical study: "Autologous Bone Marrow Cell Infusion Therapy (ABMi Therapy) for Decompensated Liver Cirrhosis Patients"

A clinical study called "Autologous Bone Marrow Cell Infusion (ABMi) Therapy for Decompensated Liver

Cirrhosis Patients" was started in November 2003, building on basic studies with the mouse GFP/CCl₄ model. Details of this clinical study, including indications, are shown as follows:

Treatment indications:

- 1) Total bilirubin: ≤ 3.0 mg/dl
- 2) Platelet count: $\geq 5.0 \times 10^{10}/l$
- 3) Good control of esophagogastric varices and hepatocellular carcinoma
- 4) Good cardiopulmonary function, and no serious comorbidities
- 5) No presence of viable hepatocellular carcinoma on computed tomography (CT), magnetic resonance imaging (MRI), or other diagnostic imaging modalities

Protocol:

Autologous bone marrow cells (400 ml) were collected under general anesthesia, and the collected bone marrow fluid was concentrated and washed. Bone marrow mono-

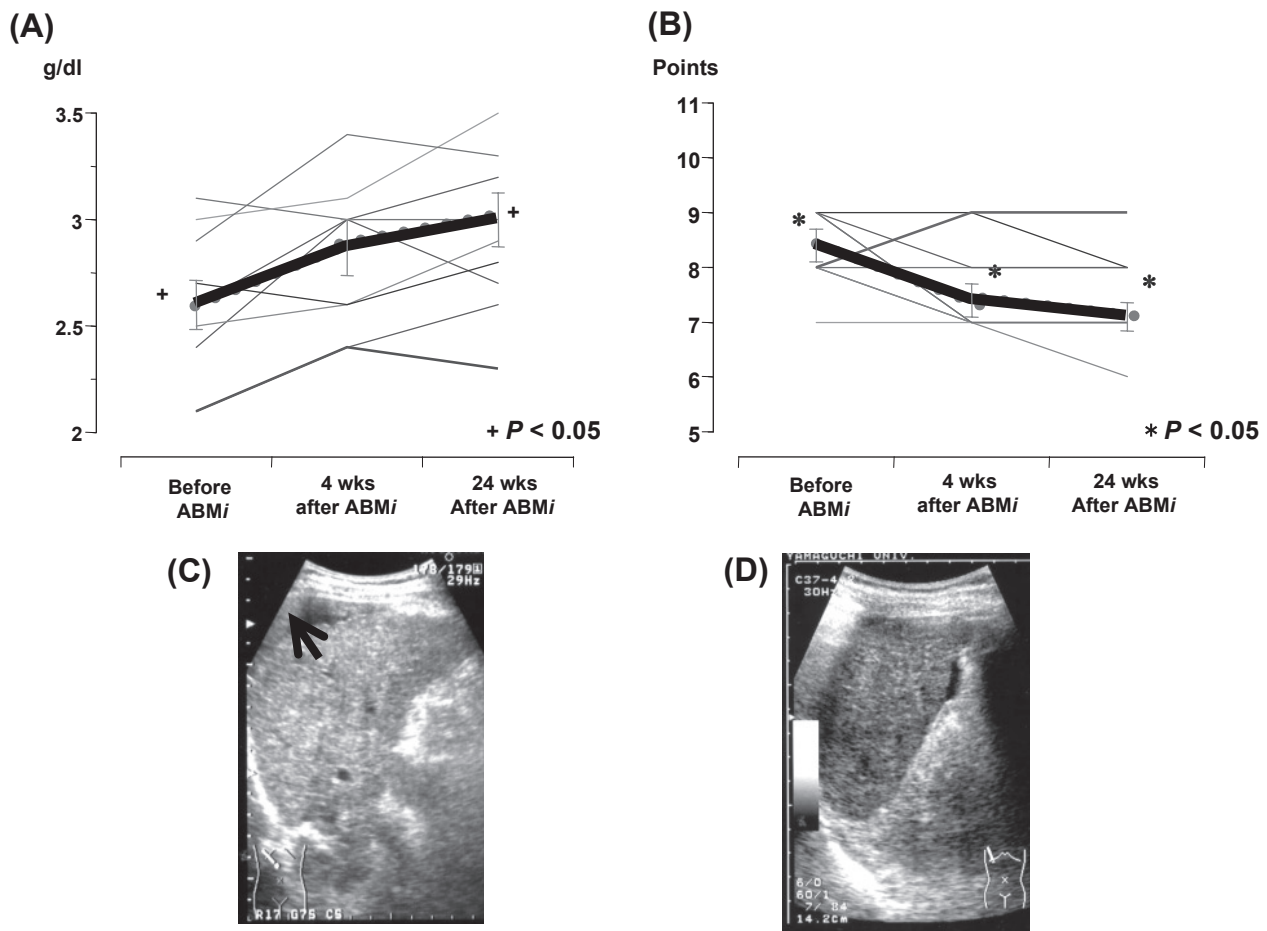


Fig. 2. Liver function improvements in liver cirrhosis patients after autologous bone marrow cell infusion (ABMi). A: Serum albumin level (g/dl). The thick line represents the mean value. B: Child-Pugh score (points). The thick line represents the mean value. C and D: Abdominal ultrasound images before and after ABMi therapy, respectively. Before ABMi therapy, ascites (arrow) is seen on the liver surface (C), but after ABMi therapy, clear reductions in ascites are apparent (D). These figures are modified from Ref. 7 with permission.

nuclear cells in that fluid were then purified and condensed according to standard operating procedures (SOP) at the regenerative and cell therapy center, which is fully equipped with good manufacturing practice (GMP) grade facilities, and administered by drip infusion via a peripheral vein to the same patient. The course was observed for 6 months after ABMi, and the efficacy and safety were evaluated by blood biochemistry tests, liver biopsy, abdominal ultrasonography, and abdominal CT. During the observation period, no changes in oral medications, antiviral drugs, or other agents were seen (7).

Results:

Serum albumin levels, total protein levels, and Child-Pugh score at 6 months after ABMi were significantly improved in patients whose course could be observed for 6 months after ABMi (7). Similar improvements were also seen in 9 patients whose course could be observed for 15 months (Fig. 2). We had treated 24 patients as of October 2009, and no particular problems with occurrence of adverse events were encountered (15). Additional trials of the ABMi therapy we developed have been conducted with 6 patients at Yamagata University (conducted jointly with a Yamagata University team) and 10 patients at Yonsei University in South Korea (16). These results of our multicenter clinical trials are gradually demonstrating the safety and efficacy of ABMi therapy and therapy using autologous bone marrow cells.

4. Conclusions

Reports to date on regenerative and cell therapies using bone marrow (stem) cells for liver cirrhosis include not only those on our ABMi therapy (7, 15), but also reports from India on the effectiveness of peripheral administration of bone marrow stem cells (17, 18). Lyra et al. in Brazil also reported the feasibility and safety of autologous bone marrow cell infusion through not a peripheral vein but a hepatic artery for chronic liver disease patients waiting liver transplantation (19, 20). Moreover, Kharaziha et al. showed liver function improvements after administration of autologous mesenchymal stem cells (21). Issues to be investigated in the future include identification of cells showing treatment effects from bone marrow cell fractions and clarification of the mechanisms of such actions. When cells with liver regeneration and restorative activity can be isolated from small amounts of bone marrow fluid, cultured, and then re-administered, the indications would be able to be expanded so that collection of bone marrow fluid would no longer need to be performed under general anesthesia. However, safety evaluation guidelines for cultured cells are needed when autologous marrow-derived cultured

cells are used in patients, and a system conforming to SOP at a cell-processing center with GMP grade is essential. In the future, based on liver-function improvement effects from administration of bone marrow (stem) cells for cirrhosis that have been confirmed to date, new treatment methods using less invasive bone marrow-derived cultured cells will need to be developed.

Acknowledgments

This study was supported by Grants-in-Aid for scientific research from the Japan Society for the Promotion of Science, Ministry of Health, Labour, and Welfare, the Knowledge Cluster Initiative, and Japan Science and Technology Agency.

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