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Effect of low-molecular-weight heparin on the commitment of bone marrow cells to liver sinusoidal endothelial cells in CCl_4-induced liver injury

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Abstract

Background/aims: Recently liver regeneration by bone marrow transplantation has been proposed as an alternative source of functional liver cells. We investigate commitment of bone marrow cells (BMCs) to liver regeneration and the effect of dalteparin sodium (DS) on regeneration of the damaged liver caused by carbon tetrachloride (CCl_4) administration in the mice.
Methods: Liver injury was produced in 8-week-old mice by treating with CCl_4 for 4 weeks. Thereafter, mice received a lethal dose of irradiation (10 Gy) to whole body, followed by injection of 1×10^7 green fluorescent protein (GFP)-positive BMCs via the tail vein. DS (50 IU/kg, intraperitoneally) was administered daily for 28 consecutive days starting at 1 day post-BMC transplantation. Lineage marker analysis of GFP-positive liver cells was performed immunostaining with a CD31 antibody.
Result: Four weeks after BMC transplantation, GFP-positive cells in the CCl_4-damaged liver could be detected in the lobule displaying a meshwork architecture extending from the periportal to pericentral regions, a pattern simulating sinusoidal lining. This localization of GFP-positive cells suggested that these cells were closely associated with sinusoidal endothelial cells. By staining the GFP-positive cells for CD31, it was confirmed that the majority of the GFP-positive cells are also positive for CD31. The GFP^+CD31^+ cells were barely detected in the control group (1.0 ± 1.2 per field). In marked contrast, a numerous number of GFP^+CD31^+ cells were detected in the liver section obtained from the CCl_4-induced liver damage group (3.8 ± 1.3 per field, $P < 0.05$ versus control). The number of GFP^+CD31^+ cells in CCl_4 plus DS-treated group was further increased to 8.3 ± 1.3 per field ($P < 0.05$ versus CCl_4-induced liver damage group).
Conclusion: The majority of GFP-positive BMCs was committed to sinusoidal endothelial cells. DS promoted BMC differentiation into sinusoidal endothelial cells in the CCl_4-damaged liver.

Keywords: Dalteparin sodium; Bone marrow cell transplantation; Liver regeneration; Sinusoidal endothelial cells

1. Introduction

Development of the cell therapy-based strategies for the treatment of liver failures attracts a keen attention because of the limitation of orthotopic liver transplantation, including shortage of donor livers. Recent advances in stem cell research have revealed the importance of immature cells in organ maintenance. Bone marrow cells (BMCs) have been suggested as a source for the immature cells of multi-organs [1–3]. Regarding the liver, a close relation between liver-consisting cells and hematopoietic cells has been reported.

Abbreviations: BMCs, bone marrow cells; CCl_4, carbon tetrachloride; DS, dalteparin sodium; FAH, fumarylacetoacetate hydrolase; GFP, green fluorescent protein; HE, hematoxylin eosin; HGF, hepatocyte growth factor; PBS, phosphate-buffered saline; TRITC, tetramethylrhodamine isomer R

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Liver regeneration by bone marrow transplantation has been investigated as other alternative sources of functional liver cells [4–6].

Transdifferentiation of BMCs into a mature cell form is not limited to the lineage of parenchymal cells. Indeed, it has been suggested that BMCs can be transdifferentiated into non parenchymal cells including endothelial cells and mesenchymal cells [4,7]. Hepatic sinusoidal endothelial cells (SECs) contribute to regulate hepatic microcirculation and immune response. The SECs are very susceptible to insults of liver damage such as hypoxia, playing an important role in liver pathophysiology [8,9]. Moreover, recovery of damaged liver requires the restoration of integrity of SECs. To date, only few reports investigate the transdifferentiation of bone marrow into SECs [4]. Thus, it is of importance to develop a therapeutic strategy to enhance SEC reconstruction.

On the other hand, heparin is widely used as a general anticoagulant, and has been recently reported to elevate plasma hepatocyte growth factor (HGF) levels [10], and heparin treatment accelerated liver regeneration [11,12]. HGF was shown to promote regeneration of SECs [13]. However, heparin has a potent prohemorrhagic property that may exacerbate severe liver injury. Dalteparin sodium (DS) is a low-molecular-weight heparin with a mean molecular weight of 5000 [14]. DS elicits an antithrombotic effect through a mechanism depending on the anti-factor Xa activity but not on the antithrombin activity. These characteristics of DS are beneficial to reduce the bleeding risk of patients. In this study, we determine the commitment of bone marrow cells to SECs in CCl_4-induced liver damage and the effect of DS on BMCs commitment to SECs.

2. Materials and methods

2.1. Mice

Green fluorescent protein (GFP) transgenic mice 8-weeks old were kindly provided by Dr. Masaru Okabe (Genome Research Center, Osaka University) [15]. Female C57BL/6 mice 8-weeks old (Charles River Japan, Kanagawa, Japan) served as recipients. Mice were properly anesthetized with ether during experiments. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Juntendo University School of Medicine.

2.2. BMCs preparation and transplantation

For the isolation of bone marrow cells (BMCs), GFP transgenic mice were treated with 5-fluorouracil (150 μg/g BW) to destroy active cycling cells [16]. At 48 h after injection, mice were sacrificed by cervical dislocation, and their limbs were removed. GFP-positive BMCs were flushed with Dulbeco's Modified Eagle Medium (GIBCO, Grand Island, New York) from the medullary cavities of the femurs and tibias using a 28G needle.

2.3. Liver damage and dalteparin sodium administration

In the liver damage group, C57BL/6 mice were administered 0.2 ml/kg of carbon tetrachloride (CCl_4) twice a week for 4 weeks. After the completion of 4-week injections of CCl_4, mice received a lethal dose of irradiation (10 Gy) to whole body, after which 1×10^7 GFP-positive BMCs were injected slowly into the tail vein using a 31G needle. After BMC transplantation, CCl_4 injection was discontinued. In some mice, dalteparin sodium (DS) was administered daily (50 IU/kg intraperitoneally, Kissei Pharmaceutical, Tokyo, Japan) for 28 consecutive days starting at one day after BMC transplantation. As a control, 1×10^7 GFP-positive BMCs were injected into C57BL/6 females that had not been treated with CCl_4.

2.4. Tissue preparation

Four weeks after BMC transplantation, the livers were thoroughly perfused via the heart with phosphate-buffered saline (PBS) to wash out contaminating blood cells. For fixation, the perfused livers were incubated with 4% paraformaldehyde overnight, and then soaked in 30% sucrose for a few days. Tissues were frozen in powdered dry ice and then sectioned into 18-μm slices using a cryostat (LEICA CM 1900, FINETEC, Heidelberg, Germany) in preparation for dyeing.

2.5. Fluorescent microscopy and immunohistochemical staining

Cells expressing GFP were analyzed by both fluorescent microscopy and conventional immnohistochemistry. Liver tissues were washed three times with PBS, and then soaked in 10% normal goat serum for 1 h. After thorough washing, sections were incubated with an anti-GFP antibody (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h followed by incubation with tetramethylrhodamine isomer R (TRITC) goat anti Rat IgG (Santa Cruz Biotechnology) as a secondary antibody for 1 h at 37 °C. Subsequently, sections were mounted on glass slides in 1% gelatin/saline. For the detection of CD31, an identical process was carried out expect for using an anti-CD31 antibody as a primary antibody (BD Bioscience, San Jose, CA). Either GFP expression or antigen stained by fluorescence-conjugated antibody was visualized under a fluorescent microscope Axioplan 2 imaging (Zeiss, Oberkochen, Germany) with appropriate filter sets.

3. Result

3.1. Chronic liver damage resulting from the repeated injection of CCl_4

First we investigated the effect of carbon tetrachloride (CCl_4) on hepatic injury and fibrogenesis in vivo. Mice were