Biochemical and Biophysical Research Communications 412 (2011) 50–54

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

HMG-CoA reductase inhibitor augments the serum total cholesterol-lowering effect of human adipose tissue-derived multilineage progenitor cells in hyperlipidemic homozygous Watanabe rabbits

Ayami Saga [a], Hanayuki Okura [a], Mayumi Soeda [a], Junko Tani [a], Yuichi Fumimoto [a], Hiroshi Komoda [a], Mariko Moriyama [a,b], Hiroyuki Moriyama [b], Shizuya Yamashita [c], Akihiro Ichinose [d], Takashi Daimon [e], Takao Hayakawa [b], Akifumi Matsuyama [a,*]

[a] Department of Somatic Stem Cell Therapy and Health Policy, Foundation for Biomedical Research and Innovation, TRI305, 1-5-4 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan
[b] Pharmaceutical Research and Technology Institute, Kinki University, 3-4-1 Kowakae, Higashi-Osaka, Osaka 577-8502, Japan
[c] Division of Cardiology, Department of Internal Medicine, Osaka University Graduate School of Medicine, Suita, Osaka 565-0871, Japan
[d] Department of Plastic Surgery, Kobe University Hospital, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe, Hyogo 660-0017, Japan
[e] Division of Biostatistics, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya, Hyogo 663-8501, Japan

ARTICLE INFO

Article history:
Received 7 July 2011
Available online 22 July 2011

Keywords:
ADMPC
WHHL
HMG-CoA reductase inhibitor
Pravastatin
Cholesterol
Cell therapy

ABSTRACT

Familial hypercholesterolemia (FH) is an autosomal codominant disease characterized by high concentrations of proatherogenic lipoproteins secondary to deficiency in low-density lipoprotein (LDL) receptor. We reported recently the use of *in situ* stem cell therapy of human adipose tissue-derived multilineage progenitor cells (hADMPCs) in lowering serum total cholesterol in the homozygous Watanabe heritable hyperlipidemic (WHHL) rabbits, an animal model of homozygous FH. Here we demonstrate that pravastatin, an HMG-CoA reductase inhibitor, augmented the cholesterol-lowering effect of transplanted hADMPCs and enhanced LDL clearance in homozygous WHHL rabbit. The results suggest the potential beneficial effects of *in situ* stem cell therapy in concert with appropriately selected pharmaceutical agents, in regenerative medicine.

1. Introduction

Familial hypercholesterolemia (FH) is characterized by premature and accelerated development of atherosclerotic lesions caused by elevated levels of cholesterol-rich lipoproteins in plasma. The disease is caused by mutations in the low-density lipoprotein (LDL) receptor gene that result in a significant decrease in receptor-mediated uptake of lipoproteins from the circulation [1–3]. Patients homozygous for defects in LDL receptors have serum cholesterol levels 5–10 times those of normal and suffer as early as the first two decades of life serious complications such as coronary artery disease [4,5]. In homozygous FH patients, conventional drug therapy such as HMG-CoA reductase inhibitors, collectively known as "statins", cannot treat the condition, and therapeutic recourses are limited to chronic plasmapheresis and orthotopic liver transplantation [1]. Although liver transplants lower LDL levels, the procedure is life threatening and, in addition, donor livers are in short supply. A number of gene therapy approaches have shown some promise in animal models and human [6–9]. As an alternative to whole-organ transplantation and/or gene therapy, cellular transplantation has been proposed to provide functional LDL receptors for the treatment of hypercholesterolemia. Transplantation of allogenic and xenogenic hepatocytes is reported to be effective in lowering serum cholesterol in the Watanabe heritable hyperlipidemic (WHHL) rabbit [10–13], which is an animal model of homozygous FH. In this context, we have reported the ability of human adipose tissue-derived multilineage progenitor cells (hADMPCs) to differentiate into hepatocytes both *in vitro* and *in vivo* and to rectify critical liver functions [14,15] similar to reports from other laboratories [16,17]. Various groups have demonstrated the *in vitro* differentiation of hADMPCs into various cell types and confirmed that hADMPCs can be easily and safely obtained in large quantities without serious ethical issues [14,15,18,19]. In homozygous FH patients, HMG-CoA reductase inhibitors have no effect on the condition as mentioned [20]. We hypothesized that HMG-CoA reductase inhibitor can act in concert with *in situ* differentiated hepatocyte-like cells originating from transplanted hADMPCs to lower serum cholesterol

* Corresponding author. Fax: +81 78 304 8707.
E-mail address: akifumi-matsuyama@umin.ac.jp (A. Matsuyama).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.07.035

levels in hyperlipidemia. To test our hypothesis, we tested the effects of treatment with HMG-CoA reductase inhibitor in hADMPC-transplanted homozygous WHHL rabbits.

2. Materials and methods

2.1. Adipose tissue samples

Subcutaneous adipose tissue samples (10–50 g, each) were resected during plastic surgery in 5 females (age, 20–60 years) as excess discards. The study protocol was approved by the Review Board for Human Research of Kobe University Graduate School of Medicine, Osaka University Graduate School of Medicine, Kinki University Pharmaceutical Research and Technology Institute and Foundation for Biomedical Research and Innovation. Each subject provided a signed informed consent.

2.2. isolation of hADMPCs

The hADMPCs were prepared as described previously [21] with some modification [14,15,18,19]. Briefly, the resected excess adipose tissue was minced and then digested at 37 °C for 1 h in Hank's balanced salt solution (HBSS, GIBCO Invitrogen, Grand Island, NY) containing 0.075% collagenase type I (Sigma Aldrich, St. Louis, MO). Digests were filtered through a cell strainer (BD Bioscience, San Jose, CA) and centrifuged at 800 *g* for 10 min. Erythrocytes were excluded using density gradient centrifugation with Lymphoprep ($d = 1.077$; Nycomed, Oslo, Norway), and the remaining cells were cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO Invitrogen) with 10% defined fetal bovine serum (FBS, GIBCO Invitrogen) for 24 h at 37 °C. Following incubation, the adherent cells were washed extensively and then treated with 0.2 g/l ethylenediaminetetraacetate (EDTA) solution (Nacalai Tesque, Kyoto, Japan). The resulting suspended cells were replated at a density of 10,000 cells/cm^2 on human fibronectin (FN)-coated dishes (AGC, Tokyo, Japan) in Stem Cell Medium (Nipro, Osaka, Japan), 1× insulin-transferring selenium (ITS, GIBCO Invitrogen), 1 nM dexamethasone (Sigma–Aldrich), 100 μM ascorbic acid 2-phosphate (Sigma Aldrich), 10 ng/ml epidermal growth factor (EGF, PeproTec, Rocky Hill, NJ), and 5% FBS (GIBCO Invitrogen). After 5–6 passages, the hADMPCs were used for transplantation.

2.3. hADMPCs transplantation and immunosuppression/statin treatment regimen

The transplantation procedure was performed as reported previously [15]. Briefly, 8-week-old homozygous WHHL rabbits (Kitayamalabes, Inc., Japan) ($n = 7$) were anesthetized with pentobarbital (50 mg/kg) and an incision distal and parallel to the lower end of the ribcage was made. The peritoneum was incised and hADMPCs (3×10^7 cells) suspended in 3 mL of HBSS (20 °C) with heparin were infused within 5 min into the portal vein via a 18-gauge Angiocath™ (BD, UT) (Fig. 1A). The immunosuppression regimen (Fig. 1B) consisted of the following: (i) intramuscular injection of cyclosporin A (6 mg/kg/day) daily from the day before surgery to sacrifice; (ii) intramuscular injection of rapamycin (0.05 mg/kg/day) daily from the day before surgery to sacrifice; (iii) methylprednisolone at 3 mg/kg/day (day −1 to 7), followed by tapering to 2 mg/kg/day (day 8–14), 1 mg/kg/day (day 15–21) and 0.5 mg/kg/day (day 22 to sacrifice); (iv) intravenous injection of cyclophosphamide (20 mg/kg/day) at day 0, 2, 5 and 7; (v) intramuscular injection of ganciclovir (2.5 mg/kg/day) was also administrated to avoid viral infection in the immunocompromised host. Twelve weeks after hADMPCs transplantation, the rabbits were divided into two groups; the first was treated with low dose pravastatin (0.75 mg/kg/day i.m., $n = 4$), an HMG-CoA reductase inhibitor (treatment group), while the second served as the control and injected intramuscularly with the vehicle ($n = 3$).

2.4. Assay for lipid profiling

Serum samples were obtained from nonfasting rabbits before and after pravastatin treatment (at 12 and 16 weeks). Serum total cholesterol and HDL-cholesterol fraction were measured using assay kits from Wako Pure Chemical Industries (Osaka, Japan)

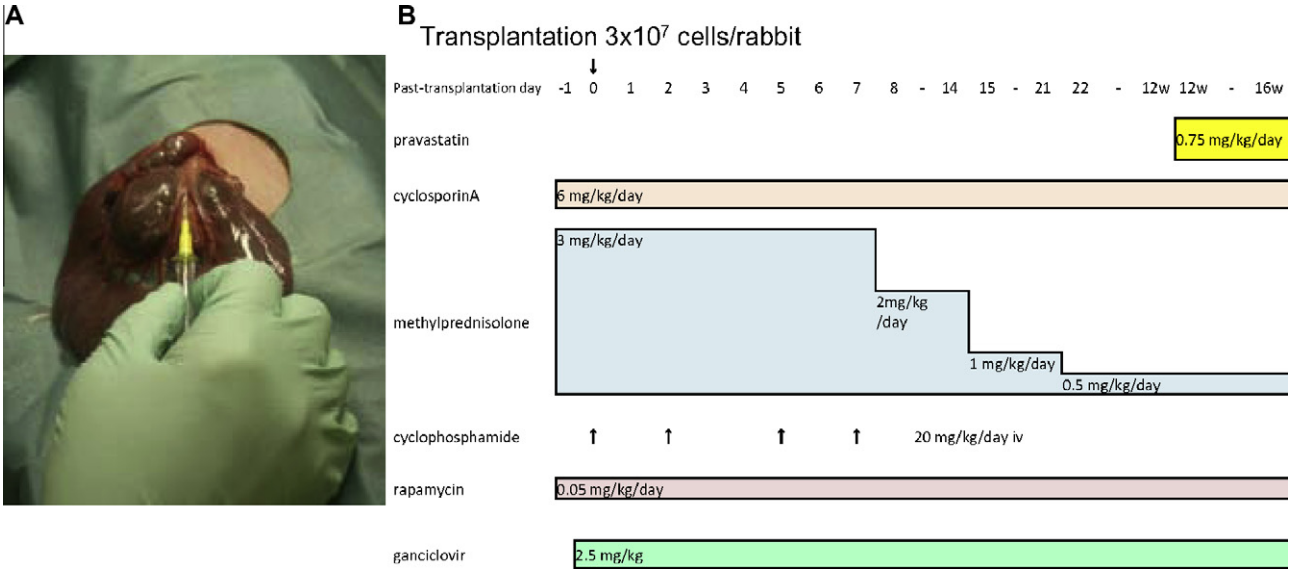

Fig. 1. (A) Surgical procedure. Watanabe heritable hyperlipidemic (WHHL) rabbits were anesthetized with pentobarbital. An incision was made distal and parallel to the lower end of the ribcage. The peritoneum was incised and hADMPCs (3×10^7 cells/rabbit) were infused into the portal vein using an 18-gauge Angiocath. (B) Immunosuppression regimen. Cyclosporin A (6 mg/kg/day) and rapamycin (0.05 mg/kg/day) were administered intramuscularly daily from the day before surgery to sacrifice. Methylprednisolone was administered at 3 mg/kg/day (days 1–7), 2 mg/kg/day (days 8–14), 1 mg/kg/day (days 15–21), and 0.5 mg/kg/day (day 22 to sacrifice). Cyclophosphamide (20 mg/kg/day) was injected intravenously at days 0, 2, 5, and 7. Ganciclovir (2.5 mg/kg/day) was also injected intramuscularly to avoid viral infection in the immunocompromised host. Twelve weeks after hADMPCs transplantation, hADMPC-transplanted WHHL rabbit were divided into two groups; the pravastatin-treated group ($n = 4$) and the control vehicle group ($n = 3$).