Biochemical and Biophysical Research Communications 433 (2013) 513–518

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Effects of surface camouflaged islet transplantation on pathophysiological progression in a *db*/*db* type 2 diabetic mouse model

Jee-Heon Jeong [a], Simmyung Yook [a], Haeshin Lee [b,c], Cheol-Hee Ahn [d], Dong Yun Lee [e], Youngro Byun [a,f,*]

[a] Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 151-742, South Korea
[b] Department of Chemistry, College of Natural Science, Korea Advanced Institute of Science and Technology, Daejeon 305-701, South Korea
[c] Graduate School Nanoscience and Technology, College of Natural Science, Korea Advanced Institute of Science and Technology, Daejeon 305-701, South Korea
[d] Research Institute of Advanced Materials, Department of Materials Science and Engineering, Seoul National University, Seoul 151-742, South Korea
[e] Department of Bioengineering, College of Engineering, and Institute for Bioengineering and Biopharmaceutical Research, Hanyang University, Seoul 133-791, South Korea
[f] Department of Molecular Medicine and Biopharmaceutical Sciences, Graduated School of Convergence Science and Technology, Seoul National University, Seoul 151-742, South Korea

ARTICLE INFO

Article history:
Received 18 February 2013
Available online 21 March 2013

Keywords:
Islet transplantation
Type 2 diabetes
Surface modification
6-Arm-PEG–catechol
Anti-CD154 monoclonal antibody
Tacrolimus

ABSTRACT

To investigate the inhibition effects of pancreatic islet transplantation on the progression of obese type 2 diabetes, we analyzed the effects of surface camouflaged islet transplantation on delaying the disease progression in a *db*/*db* diabetic mouse model. Surface camouflaged islets using 6-arm-PEG–catechol were transplanted in *db*/*db* diabetic mice. The fat accumulation and toxicity in the liver, the expansion of islets in the pancreas, and the size change of abdominal adipocyte were analyzed. In addition, the blood glucose control, insulin levels and immunohistochemical staining of recovered tissues were analyzed after transplantation. Then co-administration of anti-CD154 monoclonal antibody and Tacrolimus (IT group) deterred the pathophysiological progression of obese type 2 diabetes. At day 3 of transplantation, the serum insulin concentration of IT group was increased compared to the *db*/*db* diabetic mice group. The immunohistochemical studies demonstrated that the mass of 6-arm-PEG–catechol grafted islet was preserved in the transplantation site for 14 days. Surface modification using 6-arm-PEG–catechol effectively inhibited the immune cell infiltration and activation of host immune cells when immunosuppressive drug was given to the *db*/*db* type 2 diabetes mice. Therefore, 6-arm-PEG–catechol grafted islets effectively restored the insulin secretion in islet recipients and prevented the disease progression in type 2 diabetes.

1. Introduction

Type 2 diabetes is characterized by a decrease in insulin secretion and an increase in insulin resistance [1–4]. Most obese type 2 diabetes show an insulin resistance associated with hyperinsulinemia in the early stage of the disease [5]. As the disease progresses, the functions of pancreatic beta cells are gradually deteriorated, finally developing severe insulin deficiency [6]. The morphological damage of the pancreatic islets in obese type 2 diabetes is induced by compensatory overexpression of insulin and the resultant exhaustion of beta cells in response to the increased insulin demand.

In these respects, we expected that additional exogenous insulin administration could compensate for excessive demand for insulin secretion from the beta cells, and furthermore, deter the progression of type 2 diabetes. The insulin therapy in early stage of obese type 2 diabetes had been found to improve both insulin action and secretion, thereby overcoming insulin resistance [7]. Thus, basal–bolus insulin therapy is one of the choices for regulating the blood glucose level of type 2 diabetic patients, offering a way to closely stimulate natural insulin delivery in the clinic [8,9]. However, the insulin therapy has shown many concerns with respect to hypoglycemia, patient willingness, and incompliance. Therefore, islet transplantation has been shown to be another alternative by which to properly deliver insulin to diabetic patients.

Several studies have used islet transplantation for the treatment of the type 2 diabetes in a mouse model [10–16]. Gates et al. have ameliorated the abnormalities of obese-hypoglycaemic by allogeneic implantation of islets [10,11]. They used a Millipore bag as a device for preventing graft rejection, but it had limitations in sensing glucose concentration and in maintaining islet viability [17]. Furthermore, it was reported that although MIN-6 cell transplantation decreased hyperglycemia in *db*/*db* mice over 100 days after transplantation, the transplanted MIN-6 cells had became the cancerous tissue [18]. Andersson et al. showed that intrasplenic

* Corresponding author at: Department of Molecular Medicine and Biopharmaceutical Sciences, Graduated School of Convergence Science and Technology, Seoul National University, Seoul 151-742, South Korea. Fax: +82 2 872 7864.
E-mail address: yrbyun@snu.ac.kr (Y. Byun).

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http://dx.doi.org/10.1016/j.bbrc.2013.03.015

islet transplantation was failed to cure obese-hyperglycemic mice [13]. Barker et al. claimed that islet transplantation was not suitable as a treatment for *db*/*db* mice [15]. Katsuragi et al. examined that transplanted islet through the portal vein prevented the diabetic progression in Otsuka Long Evans Tokushima Fatty (OLEFT) rats. They reported on the possibility of transplanted islets to prevent the pancreas and mesangial matrix in the renal glomeruli from undergoing morphological changes [19]. Collectively, there were many studies reporting about the advantage of islet transplantation for deterring pathophysiological progression of type 2 diabetes.

However, it was hard to effectively deliver the insulin secreted from transplanted islets to type 2 diabetic patients because the grafted islets were easily rejected by host immune reaction. If transplanted islets would be survived for long-term periods without the immune rejection, transplanted islets would effectively delivery insulin and deter the progression of type 2 diabetes related with insulin resistance. To ameliorate these limitations in the treatment regime and to minimize the immune reaction after transplantation, and based on the previous studies, we introduced an islet surface modification technology combined with immunosuppressive medication as a treatment for type 2 diabetes [20,21]. In the previous study, surface modification of islet effectively inhibited the immune cell recognition and infiltration [22]. Particularly, the surface camouflage of pancreatic islet using 6-arm-PEG–catechol and the administration of Tacrolimus (FK506) and anti-CD154 mAb (MR-1) were very effective in preventing immune reactions against transplanted islets [20].

In this study, 6-arm-PEG–catechol grafted islets were transplanted into *db*/*db* mice with co-administration of MR-1 and FK506 to investigate the curing effect of type 2 diabetes. Furthermore, inhibition effects of this protocol on pathophysiological damage progression in type 2 diabetes were evaluated in a *db*/*db* type 2 diabetic mice model.

2. Materials and methods

2.1. Synthesis of 6-arm-PEG–catechol

Synthesis scheme was followed by previous study [20]. Briefly, six-arm-PEG–amine (1 g, MW 15 kDa) was dissolved in N-methylpyrrolidone (NMP, 10 ml) at 60 °C for 10 min. 3,4-Dihydroxyhydrocinnamic acid (DHCA, 0.8 mmol), bezotriazole-1-yl-oxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP, 0.8 mmol), 1-hydroxybenzotriazole (HOBt, 0.8 mmol) and N,N-diisopropylethylamine (DIPEA, 0.8 mmol) were dissolbed in NMP (5 ml) in separate vials. PEG and DHCA solutions were reacted at room temperature for 6 h and followed by reacting with BOP, HOBt and DIPEA until ninhydrin assay showed a negative result. The reacted materials were dialyzed (MWCO; 8 kDa) in distilled water in acidic condition (pH = 1–2) to prevent oxidation of catechol moieties, followed by lyophilization.

2.2. Surface camouflage of isolated pancreatic islet using 6-arm-PEG–catechol

Pancreatic islets were isolated from Sprague–Dawley (SD) rats (male, 8-weeks old, Orient Bio Inc., Seongnam, South Korea). Isolated islets were cultured in RPMI-1640 (Sigma) medium with 10% fetal bovine serum (FBS). The 6-arm-PEG–catechol molecules were chemically immobilized onto the surfaces [20]. After 2 days culture, HBSS (pH 8.0) containing 0.25% (w/v) 6-arm-PEG–catechol were added to the islets to chemically immobilize 6-arm-PEG–catechol onto the islet surfaces (Fig. 1A). To verify of 6-arm-PEG–catechol coverage, the fluorescein isothiocyanate (FITC) labeled 6-arm-PEG–catechol was immobilized on the surface of islets, and then, the coverage was evaluated using a laser scanning confocal microscope (LSM510, Carl Zeiss, Germany). All of the animal experiments were carried out according to the guidelines of the Institutional Animal Care & Use Committee, Seoul National University (IACUC No. SNU-070822-5).

Cell viability of islets was analyzed by Lived/Dead Viability/Cytotoxicy assay kit (Molecular Probes, Eugen, OR), Cell Counting Kit-8 (CCK-8) assay and measuring the rate of oxygen consumption (OCR) after surface modification [20].

2.3. Transplantation of 6-arm-PEG–catechol grafted islets

Six-week-old male *db*/*db* C57BL/KsJ mice (*db*/*db* group) and their lean non-diabetic heterozygous littermates, *db*/*m* mice (*db*/*m* group), were purchased from Japan SLC Inc. (Hamatsu, Japan). Spontaneously induced type 2 diabetic *db*/*db* mice were anesthetized by intraperitoneal injection of ketamine (80 mg/kg) and xylzine (16 mg/kg) after being housed for 2 weeks. The left kidney was exposed through a lumbar incision and capsulotomy was carried out at the cadual layer of the left kidney, followed by the transplantation of 500 IEQ 6-arm-PEG–catechol grafted islets (IT group). Thus, there were three following groups: *db*/*db*, *db*/*m* and IT group. Also, MR-1 (0.2 mg/mouse) was injected at 0, 2, 4, and 6 days of post-transplantation and FK506 (0.2 mg/kg) was daily injected into the recipient intrapertoneally.

2.4. Analysis of blood samples and intraperitoneal glucose tolerance test (IPGTT)

The non-fasting blood glucose (NBG) levels were measured by drawing blood from the tail veins using a portable glucometer (Super glucocoard II, Arkray, Kyoto, Japan) before and after of transplantation (at day 0, and 3 post transplantation). Insulin concentrations in serum were determined using the ELISA (rat/mouse insulin ELISA kit, Millipore, Billerica, MA). In addition, liver toxicity (aspartate aminotransferase, AST; alanine aminotransferase, ALT) was measured using FUJI DRI-CHEM 3500 (FUJIFILM, Tokyo, Japan).

In addition, IPGTT was performed to measure the glucose responsiveness at day 5 after islet transplantation. Each group was fasted for 6 h before intraperitoneal injection of 200 mg/ml glucose (200 μl/mouse) solution. Then, the blood glucose level was measured from the tail vein using portable glucometer (Super glucocard II, Arkray, Kyoto, Japan) at 0, 15, 30, 60, and 90 min after glucose injection.

2.5. Immunohistochemistry

Six-arm-PEG–catechol grafted islets were transplanted in the kidney capsule of *db*/*db* mice. At day 14 of islet transplantation, the kidney was retrieved and fixed in neutral 4% paraformaldehyde–phosphate-buffered saline, and embedded in paraffin. The tissue slides were stained with hematoxylin and eosin (H&E), anti-insulin (Abcam, Cambridge, MA), anti-$CD4^+$ (Abcam Inc., Cambridge, MA), anti-$CD8^+$ (BioLegend, San Diego, CA), and anti-$CD20^+$ (Santa Cruz Biotechnology Inc., Santa Cruz, CA) antibodies, respectively. At day 14 of islets transplantation, the pancreas, kidney, liver, and abdominal adipose tissue were stained with H&E and their morphological changes were evaluated. The sizes of adipocyte and pancreatic islet were also measured using the ACT-2U imaging software (Nikon, Tokyo, Japan). Three slides of each group were analyzed.

2.6. Statistical analysis

All the data were expressed as mean ± SEM. Statistically analysis was carried out using unpaired *t*-test or ANOVA one-way test. A