Islet transplantation promotes podocyte regeneration in a model of diabetic nephropathy

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Background/aim: The purpose of this study was to observe whether islet transplantation could induce glomerular parietal epithelial cells to express podocyte proteins in a rat model of streptozotocin-induced diabetic nephropathy (DN).

Materials and methods: A total of 18 rats were given single injections of streptozotocin to induce a DN model. Eight weeks after the modeling, successfully established DN rats were divided into three groups: an untreated group (DN group), an islet-transplanted group (IT group), and an insulin group (IN group). The islets cells were isolated from donor rats and surgically transplanted from under the kidney capsule in the IT group. Four weeks after treatment, pathological changes in the kidney were observed by pathological staining and electron microscopy. Immunohistochemical staining for PAX-2, Ki-67, and synaptopodin was performed to evaluate cell proliferation in the kidney tissues.

Results: After 4 weeks of treatment, islet transplantation significantly alleviated damage to the podocytes and increased the number of glomerular transition cells compared to the DN and IN groups, which were defined as cells that double-stained for PAX-2 and synaptopodin in membranous nephropathy. The results of HE staining, PAS staining, and electron microscopy detection also showed that pathological changes were alleviated after islet transplantation.

Conclusion: IT restored the glomerular filtration barrier based on the regeneration of podocytes in the DN model rats, and this may provide a promising clinical therapeutic strategy for human diabetes mellitus.

Key words: Diabetic nephropathy, islet transplantation, glomerular parietal epithelial cell, podocytes

1. Introduction

Diabetic nephropathy (DN) is the most common cause of end-stage renal disease (ESRD) and accounts for more than 25% of all ESRD cases in China (1,2). The initiation and progression of DN is associated with podocyte injury and loss. It has been reported that the podocyte population can be restored and that this process is linked to the regression of DN (3).

Islet transplantation (IT) and pancreas transplantation are the most effective measures to counteract type 1 diabetes (4,5). It has been reported that IT can ameliorate albuminuria and alleviate damage to the kidney (6). We have previously demonstrated that IT can directly modify the barrier of glomerular filtration by ameliorating damage to the podocytes and the glomeruli basement membrane (7). However, the mechanisms that underlie how the barrier for glomerular filtration improves currently remain elusive.

Recent studies have demonstrated that podocytes can regenerate from parietal epithelial cell (PEC) progenitors in human DN and in a diabetic mouse model (8,9).

In this study, we examined whether IT induces PECs to express podocyte proteins. The amelioration of the structure and function of podocytes is a crucial factor in the process of recovery of early diabetic nephropathy. Thus, an increased focus on podocytes may be beneficial in the treatment of DN in clinical practice.

2. Materials and methods

2.1. Animals

This experimental study was conducted at Wenzhou Medical University. Thirty-six male Sprague Dawley (SD) rats at 8 weeks of age (body weight: 200–220 g) were obtained from the Laboratory Animal Center of Wenzhou Medical University. Eighteen of these rats were used to establish the DN models and the others were used as donor rats. All rats were housed with a 12:12-h light/dark cycle.
at 24 ± 1 °C and provided water and food ad libitum. All animal procedures were based on international guidelines and were approved by the Wenzhou Medical University Animal Policy and Welfare Committee.

A rat diabetic model was induced by a single peritoneal injection of streptozotocin (55 mg/kg) in sodium citrate buffer (pH 4.5) after overnight fasting. Plasma glucose levels were detected using drops of tail vein blood and a glucometer (Roche, Indianapolis, IN, USA). One week later, when the nonfasted blood glucose level was ≥16 mmol/L for more than 2 consecutive days, the rat diabetic model was considered to be successfully established.

At 8 weeks after diabetes induction, the rats were randomly divided into the following four groups: an untreated group (DN group, n = 6), in which the rats were left untreated; an islet-transplanted group (IT group, n = 6) that underwent islet transplantation under the kidney capsule; an insulin treatment group (IN group, n = 6), in which the rats were given insulin via subcutaneous injection of streptozotocin (55 mg/kg) in sodium citrate buffer at 0900 and 2100 hours every day (each injection contained 3–8 U); and a control group (NC group, n = 6) that consisted of nondiabetic rats.

### 2.2. Islet transplantation

Islets were isolated from the rat pancreas using the previously described methods. Briefly, the rats were anesthetized with chloral hydrate and a laparotomy was performed. After locating and ligating the entrance of the common bile duct and purification with Histopaque-1119 (0.8 mg/mL, dissolved with Hanks solution) into the intestine, the islets were harvested by a reverse perfusion of 8 mL of collagenase V (0.8 mg/mL, dissolved with Hanks solution) into the common bile duct into the intestine, the islets were fulfill incubated overnight in cell culture medium with 10% fetal bovine serum at 37 °C in 5% CO₂.

### 2.3. Tissue sampling and staining

The nonfasting blood glucose levels of all groups were measured twice per week throughout the experimental period. Four weeks after islet transplantation, all rats were humanely sacrificed and the tissue samples were harvested. The renal tissues were placed in 4% paraformaldehyde solution and 2.5% glutaraldehyde for histopathological analyses.

The kidney tissues were embedded in paraffin and cut into 4-μm sections for measurement. The sections were dewaxed with xylene, and the endogenous peroxidase was blocked with 3% hydrogen peroxide. Heat-mediated antigen retrieval was performed with a citrate buffer (pH 6.0) before commencing with the IHC staining protocol. The sections were then blocked for 10 min with 5% goat serum (Boster, Hubei, China). Indirect immunoperoxidase staining of the tissue sections was performed and the samples were incubated overnight with primary antibodies for PAX-2 and Ki-67 (Abcam, Shanghai, China) at 4 °C. The tissue sections were then washed with phosphate-buffered saline (PBS, 0.01 M), incubated with secondary antibodies, and visualized with diaminobenzidine (DAB, brown color, ZSGB-BIO, Beijing, China) and hematoxylin counterstaining under a microscope. More than 10 fields in each section of each group were evaluated. The mean value was used and the scoring was performed on blinded slides.

### 2.4. Double immunohistochemistry staining

The paraffin tissue sections were dewaxed in xylene and rehydrated in a gradient of alcohol solutions. The sections were then washed with distilled water and the endogenous peroxidase was blocked with 3% hydrogen peroxide. Heat-mediated antigen retrieval was performed with citrate buffer at pH 6 before commencing the staining protocol. The sections were then blocked for 10 min with 5% goat serum. Indirect immunoperoxidase staining of the tissue sections was performed, and the samples were incubated overnight with primary antibodies for PAX-2 and synaptopodin at 4 °C. The tissue sections were subsequently rewarmed at 37 °C and incubated with antirabbit IgG-FITC and antimouse IgG-Cy3 secondary antibodies. After staining the nuclei with Hoechst staining solution, the sections were visualized and measured under a fluorescence microscope. More than 10 fields in each section of each group were evaluated. The mean value was used and the scoring was performed on blinded slides.

### 2.5. Transmission electron microscopy

The renal cortex tissue was cut into small pieces and fixed in the glutaraldehyde solution. The renal tissues were then washed with PBS and fixed with 1% osmium tetroxide. After dehydration in an acetone gradient and embedding with Araldite M (Sigma-Aldrich), the tissues were stained with uranyl acetate and lead citrate (10). The microstructures were detected under a Philips 301 electron microscope. The changes in the podocytes were measured and assessed with Image-Pro Plus 6.0 image analysis software.

### 2.6. Statistical analysis

We used a freehand line selection tool to draw a line along the Bowman's basement membrane (BBM) and to draw around the glomerular tuft. BBM length and glomerular tuft area results were then acquired from calculations of the pixel numbers with Image J (National Institutes of Health, Bethesda, MD, USA). All of the statistical data were analyzed using SPSS 19 (IBM Corp., Armonk, NY,
USA) and the values were expressed as means ± standard deviations. Multiple comparisons between groups were calculated with one-way ANOVA. A nonparametric statistical method (chi-square test) was also used in this study. The differences between groups were considered to be significant at P < 0.05.

3. Results

3.1. Blood glucose concentrations levels in each group
In this study, the level of blood glucose (BG) in the DN group was 28.6 ± 0.58 mmol/L. Compared to the DN group, the BG levels of the IT and IN groups clearly decreased. The IT group was able to more effectively control the BG level than the IN group (7.02 ± 0.35 mmol/L versus 10.28 ± 1.17 mmol/L, respectively; P< 0.01).

3.2. Pathological staining of the renal tissues of the different groups
As demonstrated in Figure 1, periodic acid-Schiff staining (PAS, Figure 1A) and hematoxylin-eosin (H&E) staining (Figure 1B) of the renal tissues were performed to observe the histopathological changes in the tubules and glomeruli of each group. The results of PAS and H&E staining revealed that the glomerular capillary loops and local basement membranes were significantly thickened in the DN group and IN group; mesangial matrix hyperplasia was also evident. However, in the IT group, the glomerular capillary loops were thin and transparent, and there was no evident thickening of the basement membrane. Compared to the IN and DN groups, the pathological changes were significantly improved in the rats in the IT group, and the renal damage caused by diabetic nephropathy was ameliorated after islet transplantation.

3.3. Damage to the podocytes in each group
IT was an effective strategy for recovering the glomerular filtration barrier in the DN rats. Via electron microscopy, we have previously reported that IT can modify podocyte depletion with foot process fusion in DN rats (Figure 2A).

3.4. Number of cells expressing PAX-2 protein along the BBM in each group
It has been reported that the PAX-2 protein is normally restricted to PECs in the glomerulus (11). PAX-2 immunostaining was observed in all groups (Figure 2B). The cells expressing PAX-2 along the BBM were significantly increased in the DN group compared to the control group (24.15 ± 1.21 versus 1.83 ± 0.18/mm, respectively; P < 0.01). The number of cells that expressed this protein in the IT group was significantly increased compared to the DN group (39.03 ± 1.33 versus 24.15 ± 1.21/mm, respectively; P < 0.01). The average number of PAX-2-expressing cells was not different between the DN and IN groups (P > 0.05).

3.5. Number of cells expressing the Ki-67 protein along the BBM in each group
To confirm and compare the effects of IT on the proliferation of PECs along the BBM in the DN rats, Ki-67 immunostaining was performed (Figure 2C). The protein expression of Ki-67 along the BBM was rarely found in the normal rats. However, in the IN group, the expression of Ki-67 was increased compared to that of the DN group (8.77 ± 0.58 versus 6.5 ± 0.38, respectively; P < 0.01), while it was significantly lower compared to the IT group (8.77 ± 0.58 versus 19.10 ± 1.06, respectively; P < 0.05).

Figure 1. Pathological staining of the renal tissues was performed to observe the histopathological changes in the tubules and glomeruli of each group: a) PAS staining in each group (original magnification 400×); b) H&E staining in each group (original magnification 400×).
3.6. Number of glomerular cells expressing PAX-2 and synaptopodin in each group
To determine whether IT increased the numbers of glomerular epithelial transition cells (defined as cells in the glomeruli that expressed both PEC and podocyte-specific protein), double immunostaining was performed for PAX-2 and synaptopodin (Figure 2D). IT significantly increased the number of transition cells compared to the IN and DN groups (1.65 ± 0.19 versus 0.5 ± 0.10; P < 0.01 and 1.65 ± 0.19 versus 0.4 ± 0.09, respectively; P < 0.01).
4. Discussion
Over the past few years, islet transplantation has emerged as a promising therapeutic method in the use of conventional antidiabetic drugs and insulin for the treatment of diabetic patients. Although we have previously reported that IT can improve the damage done to podocytes by inhibiting the TGF-β1 pathway, the current study was designed to determine whether IT could induce the expression of podocyte proteins by parietal epithelial cells. The data from our study demonstrated that proliferation of PECs along the BBM and the numbers of glomerular transition cells expressing both PEC- and podocyte-specific proteins were increased in the DN rats after transplantation.

Podocytes are highly differentiated epithelial cells in the glomeruli that are attached to the glomerular basement membrane (GBM) to maintain an efficient glomerular filtration mechanism (3,12,13). Their interdigitating foot processes are bridged by slit diaphragms and control patency via transcytosis clearance mechanisms. Studies have demonstrated that decreases in podocyte numbers and densities are critical determinants for the development of proteinuria and progressive glomerulosclerosis in DN (14,15). It has been reported that the glomerular PECs lining Bowman’s capsule can function as progenitor cell niches for podocytes and, in several forms of glomerular disease, these cells can proliferate and transdifferentiate into podocytes (16). The recovery of podocytes is a crucial factor in the process of treating early DN. Thus, podocytes may be a promising target in treating clinical DN. In recent years, remarkable clinical human results have been achieved in the application of islet transplant. In addition, researchers have shown that the kidney graft survival rates for uremic patients with diabetes mellitus can be significantly improved after islet transplant. The results of this study have demonstrated that the numbers of transitional cells expressing both the PEC phenotypic marker PAX-2 and the podocyte marker synaptopodin were increased in the IT group, and these cells were only rarely found in the IN and DN groups. These results demonstrate that islet transplantation can significantly ameliorate the thickening of the GBM and podocyte damage with fusion of the foot processes.

However, the precise mechanisms underlying how DN might induce PECs to begin to express podocyte protein is unclear. Several studies have suggested that hyperglycemia can promote podocyte apoptosis in the early stages, which consequently induces PECs, along with Bowman’s capsule activation, and proliferation and transdifferentiation of podocytes (3,17,18). PEC activation might be mediated via several transduction pathways that include Notch, Wnt/β-catenin, and HB-EGF/EGFR (19–21). In this study, we found that the activation and proliferation of PEC cells were clearly increased in the hyperglycemic state. However, there were more glomerular transition cells in the IT group than in the other groups. We hypothesize that long-term high BG leads to the induction of the actions of fibrotic factors and cytokines, such as TGF-β1, HGF, and CTGF. These overexpressions persistently promote podocyte and PEC apoptosis and hamper the differentiation of PECs into podocytes that repair damage in advanced DN. Normal control of BG after IT has been verified in earlier studies (22,23). The results of the present study also indicate that IT exhibited increased benefits in terms of repairing glomerular structure and kidney fibrosis, more so than insulin therapy in DN model rats.

The results of this study also demonstrate that islet transplantation could ameliorate podocyte damage in early DN model rats. The recovery of impaired podocyte function and microstructure is the key to treating diabetic nephropathy. In this study, islet transplantation under the kidney capsule significantly alleviated the impaired microscopic damages of podocytes in DN rats. These effects may be based on several signaling pathways during the process of development and recovery of podocytes. To the best of our knowledge, this is the first demonstration that IT restores the glomerular filtration barrier through the regeneration of podocytes in DN rats. These findings may provide a new therapeutic method or preventive treatment for early human DN and other complications related to diabetes mellitus in the future.

References


