ISSN: 3079-6601

Effects of islet organoid transplantation combined with *Tripterygium wilfordii* polysaccharide on blood glucose and urinary albumin in rats with diabetic nephropathy

Haijian Wu[1], Xin Liu[1*], Yiming Wang[1], Demin Meng[1], Jianhao Liao[1], Xinxin Chen[1]

[1]Faculty of Chinese Medicine Science Guangxi University of Chinese Medicine, Nanning, 530222。

First Author: Haijian Wu （3519551171@qq.com）

Corresponding Author: Xin Liu （694789947@qq.com）

Abstract

Objective: To explore the effects of islet organoid transplantation combined with intragastric administration of *Tripterygium wilfordii* polysaccharide on fasting blood glucose (FBG) and the urinary albumin-to-creatinine ratio (UACR) in rats with diabetic nephropathy, and to preliminarily evaluate the ameliorative effect of the combined intervention on diabetic nephropathy.Methods: Thirty-two male SD rats were selected, and a diabetic nephropathy rat model was established using a high-fat diet combined with streptozotocin (STZ). The experiment included a normal group, a model group, an islet organoid transplantation group (transplantation group), and an islet organoid transplantation combined with *Tripterygium wilfordii* polysaccharide group (combination group), with 8 rats in each group. Before transplantation, the islet organoids were identified by morphologic observation, dithizone (DTZ) staining, and Western blot analysis. The transplantation group received islet organoid transplantation, and the combination group received intragastric administration of *Tripterygium wilfordii* polysaccharide on the basis of transplantation, with continuous intervention for 8 weeks. FBG was measured in each group at weeks 0, 4, and 8 of the intervention, and UACR was measured at weeks 0 and 8; the changes in the indicators were compared among groups. Results: The islet organoids exhibited spherical islet-like structures, showed positive DTZ staining, and expressed β-cell-related proteins, indicating that they possessed an islet-like cell cluster structure and β-cell characteristics. Compared with the normal group, FBG and UACR were elevated in the model group. After 8 weeks of intervention, compared with the model group, FBG and UACR in the transplantation and combination groups showed decreasing trends, with a more pronounced decrease in the combination group, suggesting that the combined intervention may have certain advantages in improving glucose metabolic disorders and reducing urinary albumin excretion. Conclusion: Islet organoid transplantation combined with *Tripterygium wilfordii* polysaccharide led to decreasing trends in FBG and UACR in rats with diabetic nephropathy, with a better improvement trend than islet organoid transplantation alone, suggesting that the combined intervention may have potential effects in regulating blood glucose, reducing urinary albumin excretion, and alleviating diabetes-related renal injury, providing preliminary experimental evidence for the combined intervention in diabetic nephropathy; its exact efficacy and mechanisms still require further research and verification.

ISSN: 3079-6601

Keywords

islet organoid; *Tripterygium wilfordii* polysaccharide; diabetic nephropathy; urinary albumin

Introduction

Diabetic nephropathy represents a prevalent chronic microvascular complication of diabetes and a significant factor contributing to end-stage renal failure [1]. The incidence of diabetic nephropathy among patients with type 2 diabetes in China tends to be quite elevated; a meta-analysis of 30 researches indicated that the total prevalence was 21.8% [2]. Persistent hyperglycemia and the accompanying metabolic dysfunctions are intricately connected to renal hemodynamic modifications and serve as the primary link in the genesis of glomerular damage and proteinuria [1]. Consequently, mitigating renal injury while regulating blood glucose represents a vital approach for the prevention and management of diabetic nephropathy.

Islet organoids represent microtissues generated in vitro utilizing techniques like the directed differentiation of stem cells; these entities exhibit structural and functional properties of islet cells and may, to a certain degree, replicate the metabolic sensing and insulin secretion capabilities of natural islets [3]. Ongoing clinical investigations have demonstrated that transplantation of stem cell-derived islet-like tissues can enhance islet performance and glucose management in individuals with diabetes [4], indicating that islet organoids possess wide-ranging potential in cell-based therapy for diabetes.

Tripterygium wilfordii polysaccharide represents a significant bioactive constituent within the traditional Chinese medicine Tripterygium wilfordii Hook. f. Our previous research indicated that this substance can elicit pancreatic stem cells to differentiate into islet-like cell clusters comprising β cells [5]. Nevertheless, investigations into islet organoid transplantation coupled with Tripterygium wilfordii polysaccharide for the management of diabetic nephropathy remain limited, and whether this association can enhance glycemic regulation while mitigating renal damage remains unexplored.

This research utilized diabetic nephropathy-induced rats as the experimental subjects, and the impacts of islet organoid transplantation combined with Tripterygium wilfordii polysaccharide on blood glucose and urinary albumin excretion were initially assessed by monitoring FBG and UACR levels prior to and after treatment to establish a practical experimental foundation for subsequent in-depth explorations.

1 Materials and Methods

1.1 Experimental Animals

Thirty-two purebred male SD rats, weighing between 180 and 210 g, were procured from Hunan SLAC Jingda Laboratory Animal Co., Ltd. (license No.: SCXK (Xiang) 2019-0004). These subjects were kept within the Animal Laboratory of Saiensi College, Guangxi University of Chinese Medicine, under artificial light regulation with a 12-hour light/dark schedule, at a room temperature of 21–26 °C and a humidity of 50%–70%, while receiving unrestricted access to food and water, and were acclimatized for seven days. All laboratory animal management and experimental protocols followed the Guiding Opinions on the Humane Treatment of Laboratory Animals issued by the Ministry of Science and Technology of the People's Republic of China (Guokefacaizi [2006] No. 398), and were reviewed and approved by the Animal Ethics Committee of Guangxi University of Chinese Medicine (approval No.: GXTCMU-ECKS20260416-04).

ISSN: 3079-6601

1.2 Main Experimental Materials

Islet organoids were obtained from the induction culture previously established by our research group and were identified according to the methods described in Section 1.4 before transplantation. *Tripterygium wilfordii* polysaccharide was extracted and purified in our previous studies, with quality and safety profiles of its components available. The main reagents and instruments included: streptozotocin (STZ, Sigma-Aldrich, USA); a glucometer with matched test strips (Accu-Chek, Roche Diagnostics, Germany); a rat urinary albumin ELISA kit (Crystal Chem, USA); and a urinary creatinine assay kit (Nanjing Jiancheng Bioengineering Institute, China).

1.3 Grouping and Intervention

The diabetic nephropathy rats that were successfully modeled were randomly divided into a model group, an islet organoid transplantation group (transplantation group), and a combined treatment group (combination group), with an additional normal group (normal feeding); each group tentatively contained 8 rats. The model group received a matched sham operation as the control; the transplantation group received islet organoid transplantation; the combination group received intragastric administration of *Tripterygium wilfordii* polysaccharide on the basis of islet organoid transplantation, at a fixed dose verified by the preliminary experiments of our research group (reference dose: 6.25 mg/kg, once daily, for 8 consecutive weeks; the exact dose was based on the records of the preliminary experiments), without multi-dose comparison. Islet organoid transplantation was performed following the established methods of our research group. The intervention lasted for 8 weeks.

1.4 Identification of Islet Organoids

Prior to islet organoid transplantation, identification was conducted using protocols previously validated [5]. A fluorescence microscope was employed to examine the shape, dimensions, and developmental features of islet-like cell aggregates to ensure the emergence of spherical structures with distinct edges.

DTZ (dithizone) staining was executed as per protocols described in the existing research [5]: DTZ reagent was introduced into the islet-like cell aggregates. DTZ specifically binds to zinc ions in insulin secretory vesicles of β cells to form a red precipitate, and the observed staining outcomes were examined through an inverted light microscope. Cell clusters displaying iron-red coloration were deemed positive, signifying that the clusters contained β cells abundant in insulin secretion vesicles.

Quantification of betacellulin levels was performed via Western blotting. Islet-like cell cluster lysates were obtained and protein concentrations were assessed using the Bradford assay; 100 μg of total protein was subjected to 12% SDS-PAGE, electrophoretically transferred to nitrocellulose membranes, and subsequently incubated with a rabbit anti-betacellulin primary antibody overnight at 4°C. Membranes were subsequently washed with 0.05% PBST, and secondary horseradish peroxidase-labeled antibodies (1:5000) were added for 2 hours at room temperature. Following washes, specific bands were identified using ECL chemiluminescence, which was recorded for 1 to 5 minutes, with GAPDH serving as the internal standard [5].

Only islet organoids meeting these specified criteria were utilized for the transplantative trial.

1.5 Establishment of the Diabetic Nephropathy Rat Model

Based on the established high-fat diet plus STZ-induced diabetic nephropathy rat model framework, as well as methodologies presented in previous studies [6-7], a diabetic nephropathy rat platform was constructed as follows. Following one-week of homeostatic feeding, animals were supplied with a high-fat diet (consisting of 78.7% basic chow, 10% glucose, 10% animal fat, 1% cholesterol, and 0.3% sodium cholate) freely for six weeks [6].

ISSN: 3079-6601

Following overnight fasting, diabetes was subsequently triggered by a single intraperitoneal injection of STZ at 30 mg/kg [6]. At 72 hours post-injection, blood glucose concentrations were assessed from the tail vein utilizing a glucometer; repeated measurements exceeding 16.7 mmol/L indicated a successful diabetes induction [6]. Subsequently, 24-hour urine samples were gathered to evaluate 24-hour urinary protein concentrations and determine UACR; a 24-hour urine protein volume exceeding 30 mg/24 hours was deemed sufficient to fulfill the diabetic nephropathy criteria [7], and subjects failing to meet these standards were discarded.

1.6 Measurement of Fasting Blood Glucose

At weeks 0, 4, and 8 of the intervention, the fasting blood glucose level of rats in each group was measured with a glucometer after fasting, and the results were recorded.

1.7 Measurement of the Urinary Albumin-to-Creatinine Ratio

At weeks 0 and 8 of the intervention, urine samples of rats in each group were collected, and the urinary albumin and creatinine levels were measured using the corresponding kits to calculate the urinary albumin-to-creatinine ratio (UACR). UACR is an important indicator for the screening and diagnosis of diabetic kidney disease [1,8]; in this study, the change in UACR was used as the primary outcome.

1.8 Statistical Analysis

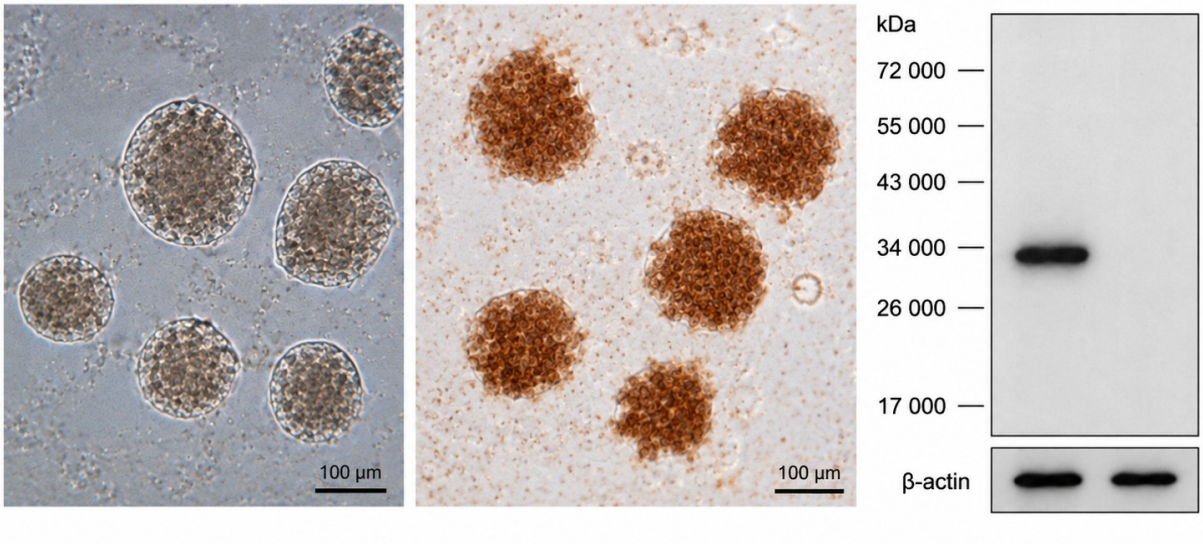
Statistical analysis was conducted utilizing SPSS and various software packages. Quantitative data were presented as mean ± SD or median (interquartile range) based on their underlying distribution. Repeated-measures analysis was employed for blood glucose measurements taken across different time intervals, while baseline-adjusted group comparisons were conducted for UACR levels. The primary focus was on distinguishing variations between the model group and the transplantation group, alongside those between the transplantation group and the combination group. Appropriate statistical techniques were chosen based on the observed data patterns, variance levels, and multiple-comparison constraints, with $P < 0.05$ deemed statistically significant. Prior to the experimental setup, the sample size justification, exclusion criteria, and blinding protocols were explicitly outlined.

2 Results

2.1 Identification Results of Islet Organoids

The islet organoids were identified before transplantation according to the methods described in Section 1.4. The results showed that, under the inverted microscope, the induced cells aggregated into spherical islet-like cell clusters with clear boundaries; after DTZ staining, the cell clusters showed iron-red positive staining, indicating that they contained β cells rich in insulin secretory granules; and Western blot analysis revealed positive expression of betacellulin. These results indicated that the islet organoids used for transplantation in this study possessed the structure of islet-like cell clusters and the functional characteristics of β cells, meeting the criteria for transplantation.

ISSN: 3079-6601



2.2 Comparison of Fasting Blood Glucose Levels Among Groups

The fasting blood glucose levels of the normal, model, transplantation, and combination groups at weeks 0, 4, and 8 were compared, and the results are shown in Table 1. After modeling, FBG was markedly elevated in each group; FBG in the normal group remained within the normal range at all time points. Compared with the normal group, FBG in the model group was significantly elevated at all time points. Compared with the model group, FBG in the transplantation and combination groups showed a decreasing trend, with a more pronounced decrease in the combination group.

Table 1 Comparison of fasting blood glucose levels among groups at different time points (mmol/L)

Group	Week 0	Week 4	Week 8
Normal group (n = 8)	5.5	5.3	5.7
Model group (n = 8)	23.6	25.2	27.1
Transplantation group (n = 8)	22.8	20.7	18.3
Combination group (n = 8)	23.1	19.6	16.2

Note: Data are shown as measured values; the corresponding statistical results are to be reported.

2.3 Comparison of UACR Levels Among Groups

The changes in UACR before and after intervention were compared among groups to analyze the effect of the combined intervention on urinary albumin excretion, and the results are shown in Table 2. Compared with the normal group, UACR in the model group was markedly elevated at both week 0 and week 8. Compared with the model group, UACR in the transplantation and combination groups showed a decreasing trend at week 8, with a more pronounced decrease in the combination group.

Table 2 Comparison of UACR levels before and after intervention (μg/mg)

Group	Week 0	Week 8
Normal group (n = 8)	32.4	31.8
Model group (n = 8)	218.3	337.1
Transplantation group (n = 8)	187.6	134.7
Combination group (n = 8)	205.7	95.2

Note: Units follow the kit specifications; measured values are shown, and the statistical results are to be reported.

ISSN: 3079-6601

3 Discussion

Islet organoids are constructed by the directed differentiation of stem cells and possess islet functional characteristics such as an endocrine cell composition and glucose-responsive insulin secretion [3]. Both animal experiments and preliminary clinical studies have shown that transplantation of stem cell-derived islet-like tissues can rebuild or improve the glycemic regulation of recipients [3-4]. In this study, the islet organoids were identified before transplantation by morphologic observation, DTZ staining, and the detection of betacellulin and other marker proteins, confirming that they possessed an islet-like cell cluster structure and β-cell characteristics [5], which provided a quality basis for their functional performance after transplantation. In this study, FBG in the transplantation group showed a decreasing trend compared with the model group, suggesting that islet organoid transplantation may improve the metabolic state of diabetic nephropathy rats by supplementing functional islet cells and enhancing insulin secretion. However, since this study did not directly detect the survival of the grafts in vivo or their insulin secretion function, the effect on blood glucose still requires further verification.

The renal-preserving impacts of Tripterygium wilfordii and its formulations have been validated by numerous investigations. Laboratory trials indicate that Tripterygium wilfordii can mitigate renal damage in diabetic mice [9]; tripterygium glycosides may alleviate glomerulonephritis and decrease urine protein levels in streptozotocin-induced diabetic nephropathy mice fed a high-fat diet [6]; triptolide may significantly decrease urine albumin levels and safeguard podocytes in diabetic nephropathy animal models [10]; and a recent Chinese internal research likewise demonstrated that tripterygium glycosides can mitigate renal damage in diabetic nephropathy mice [11]. Furthermore, investigations on the renal-preserving effects of traditional Chinese medicine therapies for diabetic nephropathy rodent models have been quite active [12]. Our earlier research verified that Tripterygium wilfordii polysaccharide can trigger pancreatic stem cells to transform into islet-like cell clusters [5]. This investigation examined Tripterygium wilfordii polysaccharide administration via gavage for islet organoid transplantation; FBG and UACR in the combined group exhibited a more downward decline than the transplantation group, implying that the integrated therapy may enhance glucose homeostasis while mitigating renal inflammation and diminishing urine albumin excretion, and may possess certain complementary clinical utility. Nevertheless, its precise outcomes still need validation through integration with renal histological and molecular-level evidence.

This study has the following limitations. First, only FBG and UACR were measured, without renal histopathological evidence, which cannot directly reflect the degree of improvement in structural renal injury. Second, the survival of the grafts in vivo and insulin secretion function were not directly detected. Third, no group with *Tripterygium wilfordii* polysaccharide alone was established, so the synergistic effects of the two interventions could not be evaluated. Fourth, molecular mechanism studies were not further conducted. These issues remain to be addressed in subsequent experiments.

4 Conclusion

This study preliminarily investigated the effects of islet organoid transplantation combined with *Tripterygium wilfordii* polysaccharide on blood glucose and urinary albumin excretion in rats with diabetic nephropathy. The results showed that FBG and UACR in the rats tended to decrease after the combined intervention, suggesting that the combined intervention may have an ameliorative effect on diabetic nephropathy. However, its exact efficacy and mechanism still require confirmation with larger samples combined with pathological and molecular evidence.

ISSN: 3079-6601

References

[1] Microvascular Complications Group of the Chinese Diabetes Society. Chinese guideline for the prevention and treatment of diabetic kidney disease (2021 edition)[J]. Chinese Journal of Diabetes Mellitus, 2021, 13(8): 762-784. (in Chinese)

[2] ZHANG X X, KONG J, YUN K. Prevalence of diabetic nephropathy among patients with type 2 diabetes mellitus in China: a meta-analysis of observational studies[J]. Journal of Diabetes Research, 2020, 2020: 2315607.

[3] YU X, LIU C, KUANG Z, et al. Islet organoids: a new hope for islet transplantation in diabetes[J]. Frontiers in Immunology, 2024, 15: 1540209.

[4] WU J, LI T, GUO M, et al. Treating a type 2 diabetic patient with impaired pancreatic islet function by personalized endoderm stem cell-derived islet tissue[J]. Cell Discovery, 2024, 10: 45.

[5] CEN Y H, LI Z H, JIA W, et al. *Tripterygium wilfordii* polysaccharide induces pancreatic stem cells to form islet-like cell clusters[J]. Chinese Journal of Tissue Engineering Research, 2018, 22(5): 729-735. (in Chinese)

[6] MA Z J, ZHANG X N, LI L, et al. *Tripterygium wilfordii* glycosides tablet ameliorates renal tubulointerstitial fibrosis via the Toll-like receptor 4/nuclear factor kappa B signaling pathway in high-fat diet fed and streptozotocin-induced diabetic rats[J]. Journal of Diabetes Research, 2015, 2015: 390428.

[7] LI L, WANG C L, MI Y, et al. Roles of podocyte-associated protein molecules in the pathogenesis of diabetic nephropathy and the interventional effects of valsartan[J]. Chinese Journal of Nephrology, 2014, 30(10): 787-789. (in Chinese)

[8] AMERICAN DIABETES ASSOCIATION PROFESSIONAL PRACTICE COMMITTEE. 11. Chronic kidney disease and risk management: standards of care in diabetes—2024[J]. Diabetes Care, 2024, 47(Suppl 1): S219-S230.

[9] CHANG B, CHEN W, ZHANG Y, et al. *Tripterygium wilfordii* mitigates hyperglycemia-induced upregulated Wnt/β-catenin expression and kidney injury in diabetic rats[J]. Experimental and Therapeutic Medicine, 2018, 15(4): 3874-3882.

[10] MA R, LIU L, LIU X, et al. Triptolide markedly attenuates albuminuria and podocyte injury in an animal model of diabetic nephropathy[J]. Experimental and Therapeutic Medicine, 2013, 6(3): 649-656.

[11] ZHANG C, SONG C D, WANG M, et al. Effects of tripterygium glycosides on renal injury in rats with diabetic nephropathy[J]. China Pharmacy, 2025, 36(7): 815-819. (in Chinese)

[12] SHEN L, YU L Q, XIONG P H. Protective effects of Liuwei Dihuang decoction combined with Huanglian Jiedu decoction on diabetic nephropathy rats and the related mechanisms[J]. Journal of Nanjing University of Traditional Chinese Medicine, 2013, 29(6): 553-557. (in Chinese)

Fund

This work was supported by the Guangxi Universities Young and Middle-aged Teachers' Basic Scientific Research Ability Improvement Project (Grant No. RZ2500000217); the 2024 University-Level Research Project of Guangxi University of Chinese Medicine — Youth Science Fund Project (Grant No. 2024QN026); the University-Level Research Project of the Faculty of Chinese Medicine Science, Guangxi University of Chinese Medicine — Natural Science General Program (Grant No. RZ2400000157); and the 2025 College Students' Innovation Training Program of the Faculty of Chinese Medicine Science, Guangxi University of Chinese Medicine (Grant Nos. S202613643024, S202613643031, S202613643060, and S202613643066).