Effect of T3 On the expression of TGF-β_1 in the mice diabetic kidney

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Abstract: **Objective** To observe the effect of triiodothyronine (T3) on the expression of transforming growth factor-β_1 (TGF-β_1), in order to investigate the mechanism of T3 in renal fibrosis. **Methods** Thirty-six C57/B6 mice were used in this study. The control group (C) included 12 mice, and the others were used to establish diabetic models by injection of streptozotocin (STZ). Then the mice were randomly divided into the diabetes group (DM) and T3 group (T3). The mice were raised for six months. The expression of TGF-β_1 was detected by immunohistochemical stainning, image analysis and Western blot. **Results** Compared with the control group, the expression of TGF-β_1 was obvi-ously increased in the diabetes group, and the expression of TGF-β_1 in the T3 group was obviously lower than that in the diabetes group. **Conclusion** T3 reduces the range and process of fibrosis in dia-betic nephropathy by down-regulating expressions of TGF-β_1.
Keywords: T3; Diabetes mellitus; Kidney; TGF-β_1

0 Introduction

In recent years, the incidence rate of diabetes (DM) has gradually increased, becoming a heavy burden on families and society. Patients with diabetes in the late stage are accompanied by a variety of complications, which seriously affect the quality of life. Diabetes nephropathy (DN) is a common complication in the late stage of diabetes. Microvascular disease and progressive fibrosis of glomerulus, renal tubules and renal interstitium are the main pathological features [1]. The pathogenesis of DN is not clear. A large number of literatures reported that the synthesis of extracellular matrix (ECM) increased, and the degradation decreased is the main influencing factor of DN. Transforming growth factor-β_1 (transforming growth factor-β_1, TGF-β_1) Promoting ECM synthesis and reducing degradation are currently recognized as the strongest fibrogenic factors.

TGF-β_1. It can also affect the expression of many downstream cytokines and the development of diabetes nephropathy [2]. Clinical data show that the level of thyroid hormone in serum of patients with diabetes nephropathy is correlated with the degree of renal fibrosis, and the expression of thyroid hormone receptor exists in renal epithelial cells and mesangial cells, which indicates that thyroid hormone plays a certain role in the development of diabetes renal fibrosis, but the specific mechanism is unknown. In this study, T3 was used to interfere with STZ induced diabetes mice, and TGF in renal tissue was observed-β_1 to explore the mechanism of thyroid hormone affecting renal fibrosis, and to provide experimental basis for the selection of drug targets for the treatment of diabetes nephropathy.

1 Materials and methods

1.1 Experimental animals

36 2-month-old C57/B6 mice were purchased from the experimental animal Department of China Medical University.

1.2 Main reagents

STZ, T3, TGF-β_1 monoclonal antibody (purchased from sigma company), Goat anti rabbit two-step detection kit (purchased from Maixin biology Co., Ltd.), PVDF membrane, ECL chromogenic solution (purchased from sigma company).

1.3 Experimental method

1.3.1 Animal model preparation and grouping

After 36 C57/B6 mice were adaptively fed for 1 week, 12 mice were randomly selected as normal control group. The remaining 24 rats were injected intraperitoneally with streptozotocin (STZ) to prepare diabetes models (85 mg/kg, twice, with an interval of 1 w). 1 before STZ injection, they were prepared with 0.5 mmol/L citric acid sodium citrate buffer). Normal control group mice were injected with equal volume of citric acid buffer according to body weight. 48 hours after the model was established, the tail vein of mice was taken, and the blood glucose value was greater than 16.7 mmol/L, which was the successful model of diabetes. Diabetes mice were randomly divided into diabetes group (DM group, n = 12) and thyroid hormone group (T3 group, n = 12). One month after modeling, T3 mice was given by gavage at the dose of 75μg/kg/D, and other mice were given equal volume buffer by gavage. All animals were fed for 6 months.

1.3.2 Animal kidney tissue sampling and specimen preparation

The mice were killed after cervical vertebra dislocation. The kidneys were quickly removed and washed with normal saline. The left kidneys of mice in each group were cut into small pieces and fixed with 4% paraformaldehyde; the right kidney tissue was quickly frozen in liquid nitrogen and stored at -80℃ for a long time for Western blot detection. The kidney tissue fixed with paraformaldehyde was dehydrated with gradient ethanol, transparent with xylene, and embedded in wax to prepare slices (thickness 5 μm) for immunohistochemical staining.

1.3.3 TGF in mouse kidney tissue-β Immunohistochemical staining of 1

After paraffin sections are dewaxed with xylene, ethanol from high concentration to low concentration is injected into water. 3% H2O2 at room temperature for 10min (Objective: to eliminate endogenous peroxidase activity), PBS washing for 5min, 3 times, high pressure repair antigen, and cooling to room temperature. Drip serum, block at room temperature for 10min, shake off the serum, and drip TGF-β_1 antibody (Rabbit anti mouse, 1:1500), 4℃, 12 h. Rewarming for 1h, dropping secondary antibody, 37℃, 30min, PBS washing for 5min, 3 times. Drop SABC reagent, 37℃, 30min, wash with PBS for 5min, 3 times. DAB color development lasts for 5min, and the color development is terminated with distilled water. Hematoxylin was counterstained for 5min, washed with running water for 30min, dehydrated with ethanol, xylene was transparent, and gum was sealed. PBS was added to the negative control group to replace the primary antibody.

1.3.4 TGF in mouse kidney tissue-β_1 Expression volume density measurement

Five kidney tissue blocks were randomly selected from each group of animals, and five positive sections (according to the number of sections) were selected at equal intervals. Five visual fields were selected from each section according to the "S" shape. The body density of TGF-β_1-positive sites was measured under a 400-fold light microscope using a grid test system. Point counting method (formula VV= ΣPx/ΣPc) calculate TGF-β_1, where VV is the volume density, Σ PX is the grid system falling in TGF-β_1 number of positive expression areas, Σ PC is the number of points of the grid system in the reference system (i.e. The whole renal tissue), and the result represents TGF-β_1 the relative size of the expression range.

1.3.5 Detection of TGF in mouse kidney tissue by Western blot-β_1 Expression

The kidney tissues of mice were crushed by ultrasonic pulverizer, then lysed with lysate at 4℃ for 12 hours. After centrifugation at 4℃ for 30min, the supernatant was taken and the protein content was measured by Coomassie brilliant blue method. Store at -80℃ after subpackaging. Every 20 μl protein 50 μg prepare the sample, add it into the vertical electrophoresis tank, and the starting voltage is 90 v. When the marker separates the target band, stop the electrophoresis, remove the rubber plate, and immerse it in the membrane transfer solution for 30min. Turn wet at 4℃ for 2 hours, and seal 5% skimmed milk powder at room temperature for 1 hour. Add primary antibody (Rabbit anti mouse TGF-β_1 antibody, 1:500), 4℃ shaking table for 12h, tbst for 5min, 3 times. Add secondary antibody (rabbit, 1:3000) shaking table for 1h, tbst for 5min, 3 times. ECL color rendering, photographing and genesnap analysis software were used to analyze the bands.

1.3.6 TGF in mouse kidney tissue-β_1. Statistical treatment of expression

All experimental data were expressed by $x \pm s$, and statistical analysis was performed by SPSS 16.0. Analysis of variance was used for comparison between groups. T-test was used for comparison between the two samples. $P < 0.05$ was significant, and $P < 0.01$ was significant.

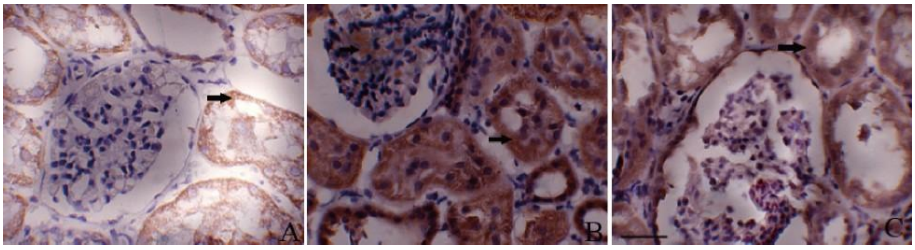

Figure 1 TGF-β_1 in kidney tissue of mice in each group Immunohistochemical staining results, scale bar = 50 μm
A. Control group (Group C); B. Diabetes group (DM group); C. Group T3

2 Results

2.1 Immunohistochemical staining results

Immunohistochemical staining results showed that TGF-β1 was positively expressed in brown granules, expressed in renal corpuscular epithelial cells and mesangial cells, renal tubular epithelial cells and tubulointerstitial cells. The expression of TGF-β_1 in the kidney tissue of mice in group C was very low (Figure 1A), and strong positive expression of TGF-β_1 was seen in the kidney tissue sections of mice in group DM (Figure 1B). The expression of TGF-β_1 was significantly attenuated (compared with the DM group), but still higher than that in the control group (Figure 1C).

2.2 Body density test results of TGF-β_1 in mouse kidney

TGF-β_1 in kidney tissue of mice in each group see figure 2 for the results of body density measurement of positive expression. From the data in the table, it can be seen that the body density value of group C is the lowest, that of group DM is the highest, and that of group T3 is lower than that of group DM but higher than that of group C, indicating that T3 can reduce TGF-β_1.

3 Discussion

Diabetes nephropathy (DN) is one of the chronic microvascular complications of diabetes. Its pathogenesis is complex. Its basic pathological characteristics are increased synthesis of extracellular matrix, thickening of glomerular basement membrane, glomerulosclerosis and renal interstitial fibrosis [3, 4]. The formation of fibrosis is a complex process involving multiple factors, and cytokine network has always been a research hotspot [5]. Several studies have shown that TGF-β_1 plays a certain role in the process of renal fibrosis in diabetes nephropathy. TGF-β_1 is mainly involved in the metabolism of extracellular matrix (ECM). On the one hand, TGF-β_1 can stimulate renal proximal convoluted tubular epithelial cells and mesangial cells to synthesize and secrete collagen; TGF-β_1 can also accelerate glucose uptake by cells and further stimulate ECM synthesis [6]. On the other hand, TGF-β_1 can reduce ECM degradation through TGF in 3 mouse kidney tissue TGF-β1 expression was analyzed by Western blot-β_1, and the value of TGF in the control group was 100-β_1 expression. The results showed that DM group had the highest content and T3 group had lower content (Figure 3).

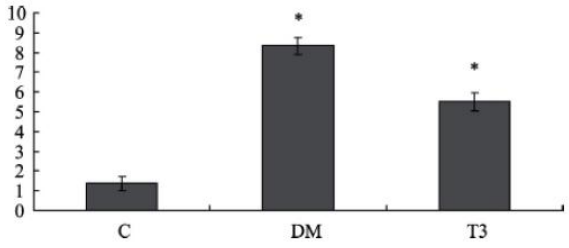

Figure 2 TGF-β_1 in kidney tissue of mice in each group Bulk density measurement results (* P < 0.01)

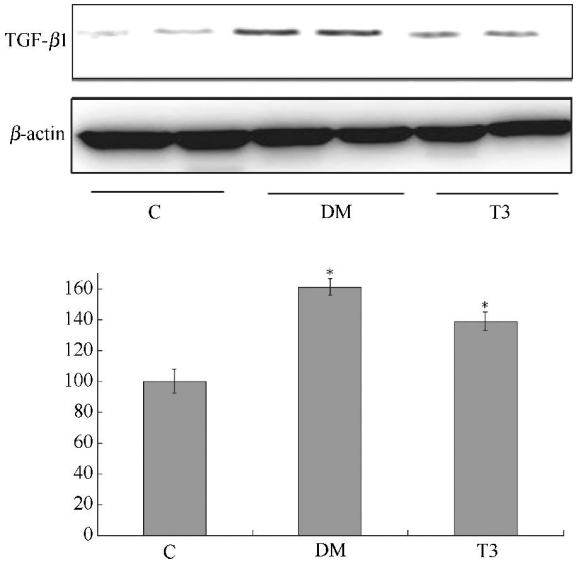

Figure 3 TGF-β_1 in kidney tissue of mice in each group Western blot results of 1 Expression

Inhibit the synthesis of matrix metalloproteinase (MMP), increase the synthesis of TIMP and reduce collagen degradation [7]. Choi et al. [8] found that exogenous TGF-β_1 promote mesangial cell TGF-β_1 mrna expression, increased collagen synthesis, mesangial cell hypertrophy, ECM accumulation, indicating TGF-β Endocrine positive feedback plays an important role in the pathological progression of DN.

Thyroid hormone has a variety of biological effects. Most of the previous studies focused on the effects of thyroid hormone on the development of nervous system. In recent years, the research direction has been expanding. Research reports that thyroid hormone receptor is expressed in renal epithelial cells and mesangial cells. Clinical data show that the serum thyroid hormone level in patients with diabetes nephropathy is abnormal, and the degree of renal fibrosis in patients with advanced stage is negatively correlated with the serum thyroid hormone level [9]. This indicates that thyroid hormone plays an important role in the formation of DN fibrosis, but the mechanism is unclear.

In this study, the expression range of TGF-β1 in the diabetic group was significantly increased by immunohistochemical staining, stereological measurement and Western blot detection, indicating that TGF-β_1 is a type of cytokine that causes DN and can promote renal tissue hypertrophy. The expression range of TGF-β_1 in the thyroid hormone treatment group was significantly weakened, indicating that thyroid hormone has an intervening effect on TGF-β_1, and can improve the state of renal fibrosis by reducing the expression range of TGF-β_1. The specific mechanism by which thyroid hormone affects the synthesis of TGF-β_1 remains to be further studied.

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