European Review for Medical and Pharmacological Sciences 2018; 22: 2483-2489

Effect of treating renal failure with decorin gene therapy

R. LI, Q.-F. WANG, W.-W. LI, X.-C. NIU

Department of Nephrology, Binzhou People's Hospital, Shandong, China

Abstract. – OBJECTIVE: To explore a new approach for treating renal insufficiency with gene therapy by implanting decorin (DCN)-expressing fibroblasts within the renal tissue of rats with renal failure to neutralize TGF-β1 activity.

MATERIALS AND METHODS: The 5/6 kidney of the selected male SD rats were removed under aseptic conditions. The rats were grouped randomly after the establishment of the model. There were 10 rats in the sham-operated group (Group A), 10 in the operation control group (without treatment, Group B), 10 in the blank control group [treated with empty vector-transfected fibroblasts (FB (LXSN) cells), Group C], and 10 in the treatment group [treated with FB (LDCNSN) cells, Group D]. The pathological changes of rats including body weight, blood lipids, renal function, and renal histology, were observed. The expression of TGF-β1 and DCN in renal tissue was detected by immunohistochemistry.

RESULTS: There were no significant differences in body weight and blood lipids between the groups at 4 weeks after treatment. The levels of blood urea nitrogen and serum creatinine in rats in Group D were significantly decreased compared with those in Group C ($p < 0.05$). Although the differences were not statistically significant, the levels of those pathological indicators are higher than baseline values. The expression of DCN in renal tissue increased significantly after 4 weeks in rats of Group D and the differences were significant compared with the other groups. There were no significant differences in TGF-β1 expression between any two groups of Group D, B, and C. Furthermore, pathological damage to the renal interstitium of rats in Group D was significantly decreased compared with that of Group B and C.

CONCLUSIONS: DCN can alleviate fibrosis and delay the progression of renal failure.

Key Words:

Fibrosis, Decorin, Transforming growth factor-β1, Gene transfection, Gene therapy, Renal interstitium.

Introduction

The clinical manifestations of kidney disease progress to kidney failure. The main pathological changes associated with renal insufficiency are glomerular sclerosis and renal interstitial fibrosis[1]. Glomerular sclerosis and renal interstitial fibrosis are closely associated with the increased expression of TGF-β1 in renal cells and infiltrating inflammatory cells[2]. Chronic renal failure is the outcome of various renal diseases and is one of the most complex problems in clinical practice. Renal damage can be caused by various causes, leading to ingravescence, severe nephron injury, and irreversible damage to the renal parenchyma[3]. When renal function declines closely to 10% of normal function, uremia occurs as a consequence of disordered metabolite excretion, electrolyte regulation, and acid-base balance[4]. There are various causes of chronic renal failure. Several primary and secondary renal diseases are continually progressive, resulting in damage to the renal parenchyma that ultimately leads to renal failure. It is generally believed that this trend is progressive and irreversible[5,6]. Immunological and gene therapies with TGF-β1 are the two focuses of research in the field of renal diseases. Decorin (DCN) is a natural antagonist of TGF-β1 that can neutralize the biological activity of TGF-β1[7]. In the present work, DCN-expressing fibroblasts (FB (LDCNSN) cells) were seeded in the renal tissue of rats with renal failure to neutralize the activity of TGF-β1. With the established renal failure model, we aimed at exploring a new approach to gene therapy for the treatment of renal insufficiency.

Materials and Methods

Experimental Animals and Grouping

Male Sprague-Dawley (SD) rats weighing from 180 to 200 g were purchased from Shanghai Slac Laboratory Animal Co. Ltd. (Shanghai, China). The study was approved by the Ethics Committee of Binzhou People's Hospital. The 5/6 kidney of the selected male SD rats were removed using a two-step method under aseptic

Corresponding Author: Xiaochun Niu, MD; e-mail: ruili160722@163.com

conditions. The rats were grouped randomly after the establishment of the model. The 10 rats in the sham-operated group (Group A) were subjected to the same surgical procedures as rats in the other groups except that both kidneys were retained. Rats in this group were allowed free access to water and food after surgery. There were 10 rats in the operation control group without injection of cells, (Group B), 10 rats in the blank control group treated with empty vector-transfected fibroblasts (FB) (LXSN), (Group C), and 10 rats in the treatment group treated with FB (LDCNSN) cells, (Group D).

The transfected fibroblasts, FB (LDCNSN) and FB (LXSN) cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 300 ng/ml G418 and 10% fetal calf serum (FCS). Cells were digested with EDTA-trypsin, and then were harvested, and washed three times with high-pressure disinfected normal saline. Cells were then resuspended and adjusted to a concentration of 1×10^7/ml. Rats in the blank control group and treatment group were subjected to 5/6 nephrectomy, followed by multi-point injection with FB (LXSN) and FB (LDCNSN) cells into the renal medulla, respectively. There were 5-6 injection sites that were uniformly distributed on the kidneys of the rates in both groups. The number of total injected cells was 1×10^6/kidney. The rats were allowed to eat and drink *ad libitum* after surgery.

Sample Collection and Observational Indexes

The rats in all the groups were subjected to body weight (BW) measurement, femoral artery blood sampling, and measurement of serum triglyceride (TG), cholesterol (Tch), creatinine (Scr), and blood urea nitrogen (BUN) with an automatic biochemical analyzer before treatment, and at 1 and 4 weeks after treatment. Renal tissue specimens were fixed in 10% formalin solution and then were preserved in liquid nitrogen.

Histological Examination

Renal tissues were embedded in paraffin and sliced into slices with a thickness of 2-3 μm. After dewaxing and staining with hematoxylin and eosin (HE), the samples were observed under a light microscope. Renal tubulointerstitial lesions were semiquantitatively graded: Grade 0: normal; Grade I: extent of disease was < 25%; Grade II: extent of disease was 26-50%; Grade III: extent of disease was > 50%[8].

Expression of DCN and TGF-β1 in Renal Tissue

The EnVision immunohistochemical staining method was used to detect changes in the expression of DCN and TGF-β1 in renal tissue. The sections of paraffin-embedded renal tissue were routinely dewaxed. The anti-TGF-β1 antibody (Amresco, Solon, OH, USA) was added to a dilution of 1:100 and anti-DCN antibody (Sigma-Aldrich, St. Louis, MO, USA) was added to a dilution of 1:400. The sections were washed at room temperature for 1 h. Signals were developed with diaminobenzidine (DAB) after adding the secondary antibody of EnVision, and sections were counterstained with hematoxylin. Sections were observed microscopically and photographed. The expression of TGF-β1 and DCN was analyzed with a semiquantitative method and the analysis was conducted simultaneously by three people. Based on the distribution of tubules within the mesenchyme, staining was divided into Grades 0-3: Grade 0: no staining; Grade 1: occasional staining; Grade 2: local lesion staining; Grade 3: diffuse staining[9].

Statistical Analysis

All data were expressed as mean ± standard deviation ($\bar{x} \pm s$). Student's *t*-test was used for comparisons. $p < 0.05$ was considered to be statistically significant.

Results

Effect of DCN Treatment on Body Weight of Rats With Renal Failure

There were no significant differences in body weight among all groups of rats before the experiments. The body weights in the four groups of rats were increased to different extents at 4 weeks after surgery, but the inter-group differences were not significant (Figure 1).

Effect of DCN Treatment on TG and Tch

The levels of blood lipids in the rats of the four groups were increased significantly after 4 weeks compared with the baseline values ($p < 0.05$) (Figure 2).

Effect of DCN Treatment on Renal Function

The levels of BUN and Scr in rats of Group D were significantly decreased compared with those in the rats of Group C ($p < 0.05$). Although

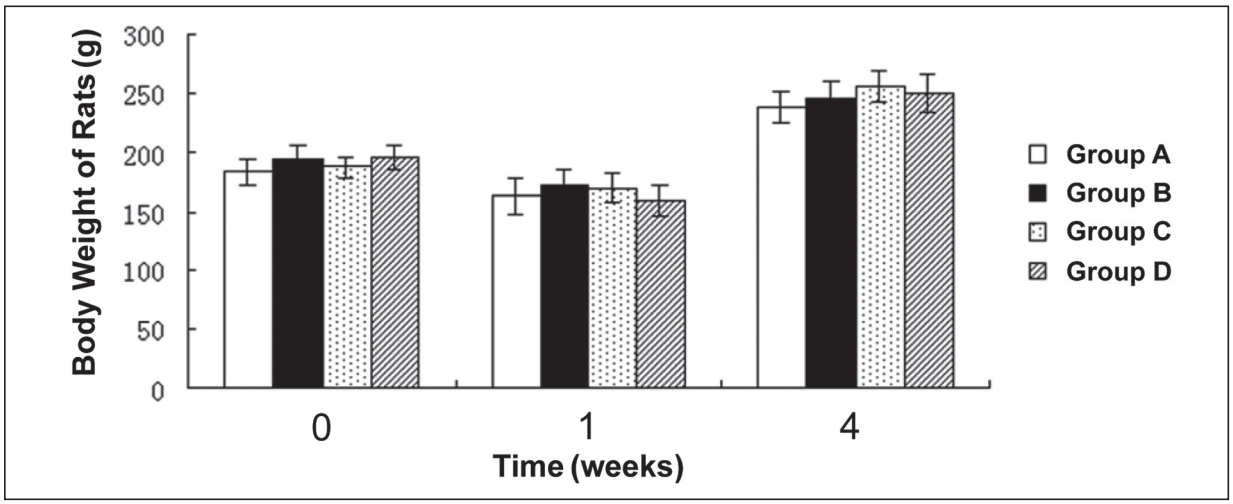

Figure 1. Changes in the body weight of rats in the different groups over time.

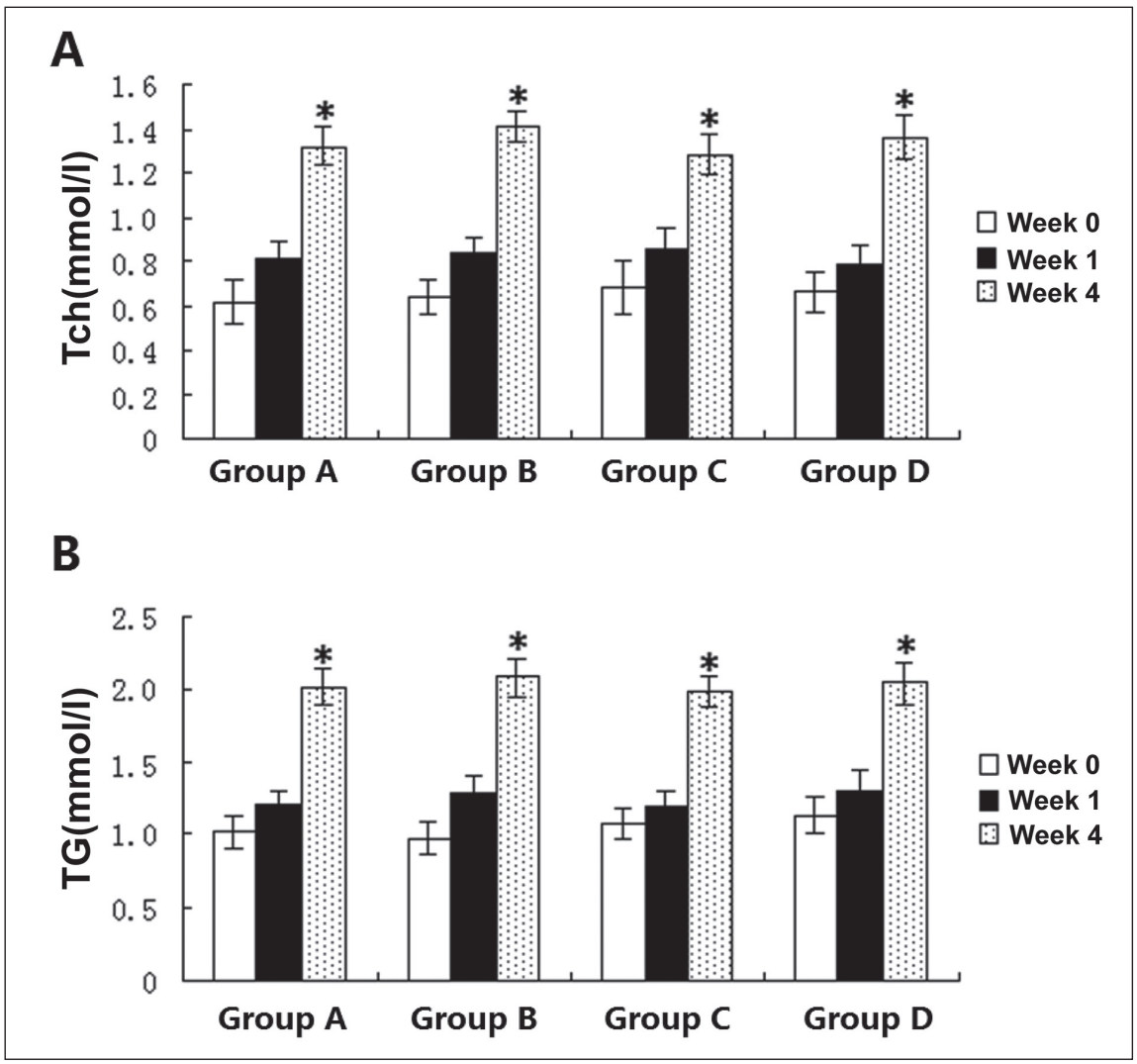

Figure 2. The levels of blood lipids in rats at the different time points.

the differences were not statistically significant, the levels of BUN and Scr were still higher than baseline values at week 0 ($p > 0.05$) (Figure 3).

Effect of DCN on Pathological Tubulointerstitial Changes, and Renal Expression of DCN and TGF-β1

The expression of DCN in renal tissue was increased significantly in rats with renal failure at 4 weeks after treatment with FB (LDCNSN) cells. Significant differences were found when compared with the other three groups ($p < 0.05$). There were no significant differences in TGF-β1 expression among the four groups (Figure 4).

Pathological Examination

The renal tissue of rats with renal failure was characterized by extensive diffuse or aggregated inflammatory cell infiltration, moderate hyperplasia of fibrous tissue, fasciculation, multiple lesions, reticular formation, partial extent of lesions over 50%, mild or moderate atrophy, and degeneration of renal tubular epithelial cells. The tubulointerstitial changes were alleviated to some extents after treatment with FB (LDCNSN) cells compared with those of the control cells (Figure 5).

Discussion

Tubulointerstitial inflammation and degree of fibrosis are closely associated with the progression of renal diseases. Renal interstitial fibrosis, which is the outcome of the combined action of various factors, is the accumulation of large

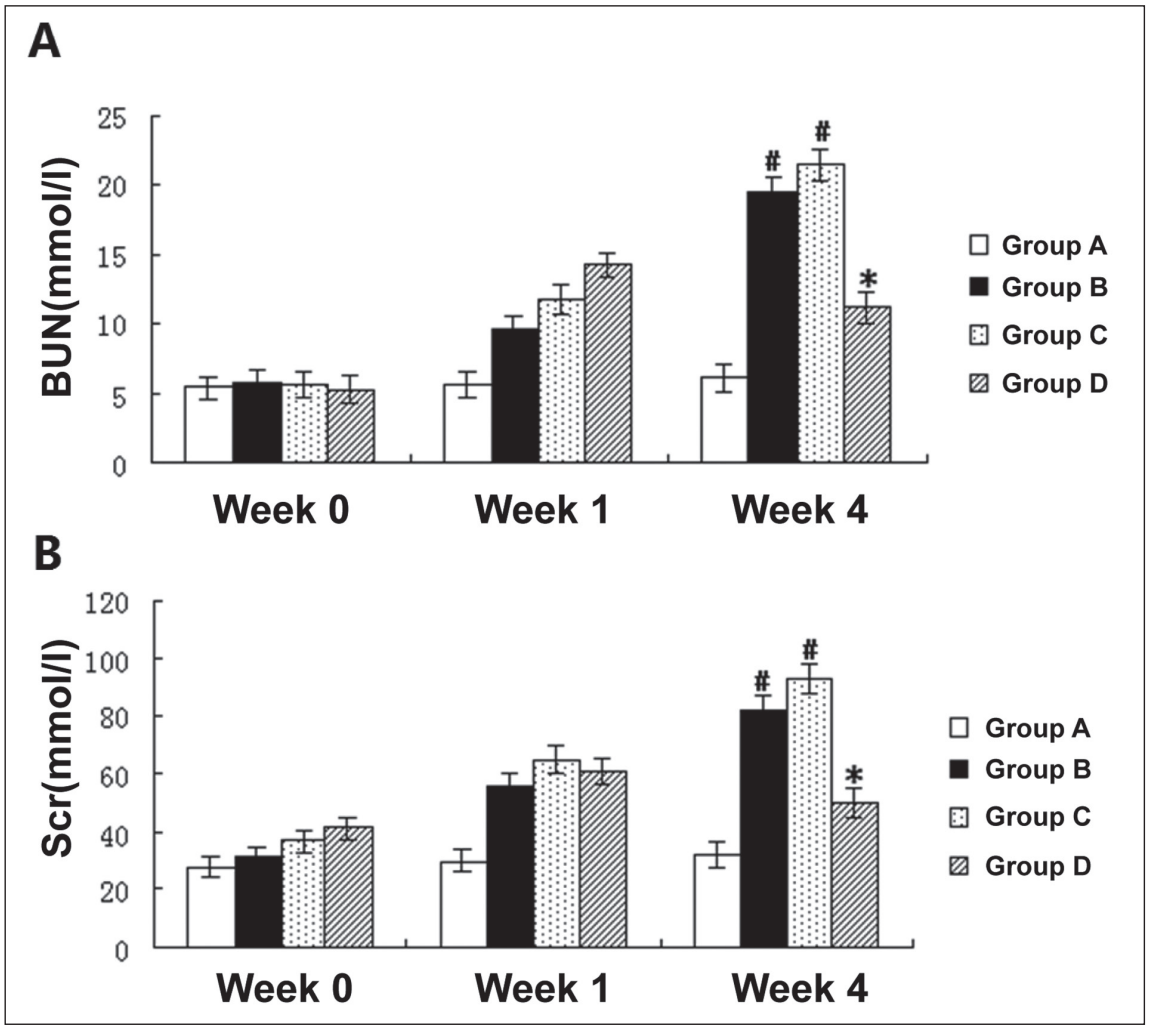

Figure 3. Renal function of rats in the different groups at the different time points.

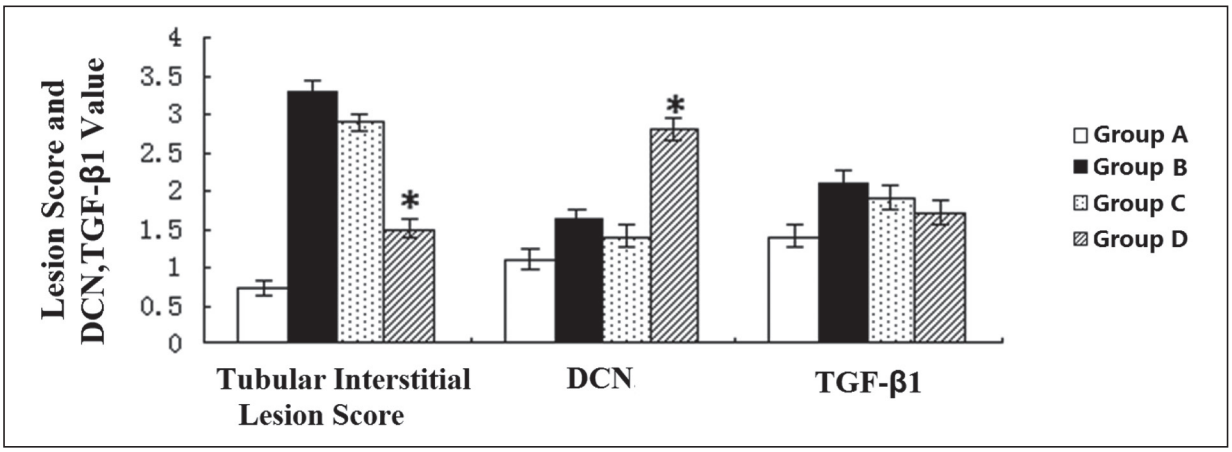

Figure 4. Tubulointerstitial changes and the expression of DCN and TGF-β1 at 4 weeks.

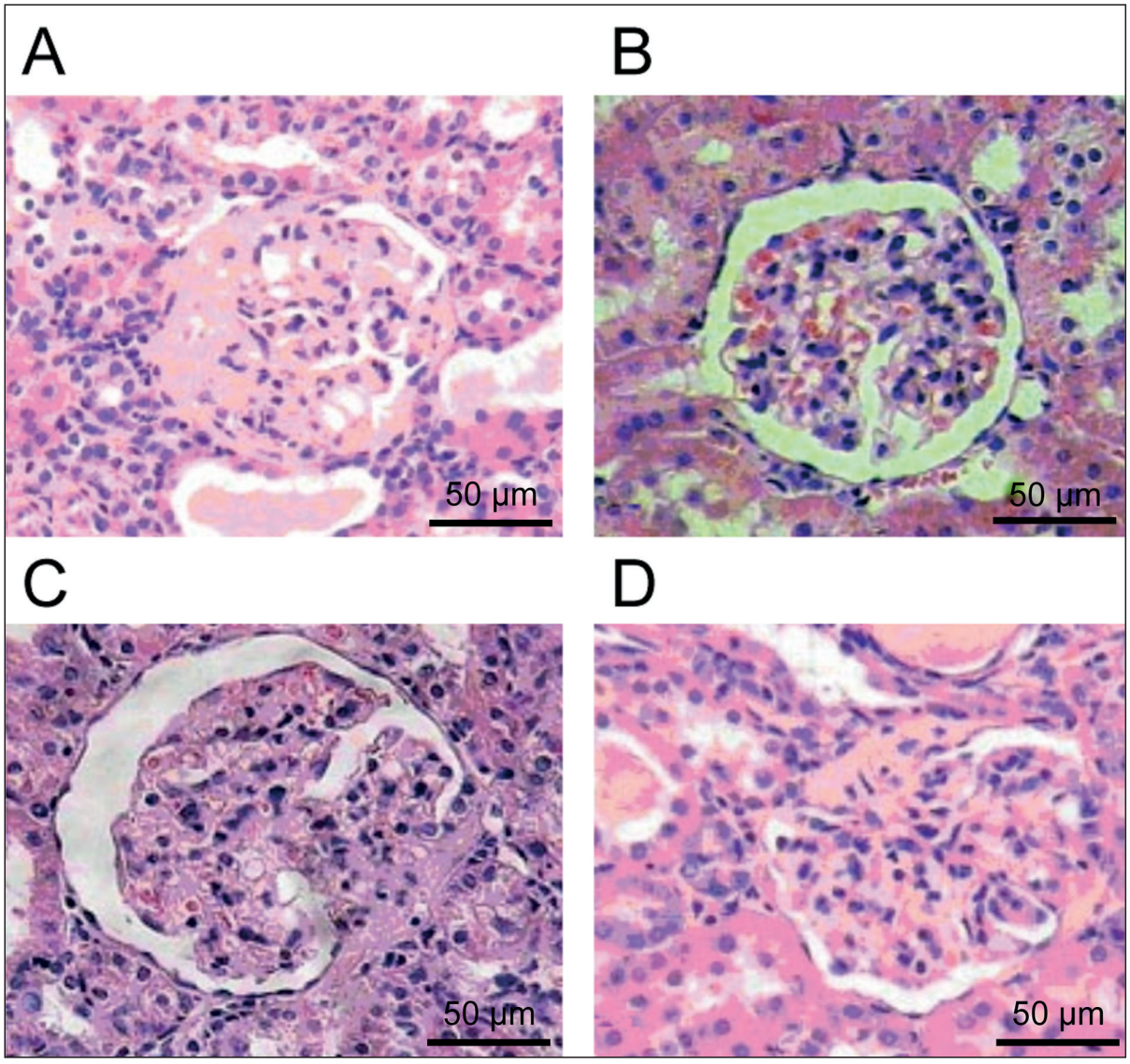

Figure 5. Pathological changes in the different groups after 4 weeks (200×).

amounts of matrix proteins within the mesenchyme and the proliferation of fibroblasts[10]. Blood lipids can affect cell proliferation, fibrosis-promoting cytokines secretion, macrophage aggregation and maturation, and other inflammation responses, and the regulation of signal transduction in cells[11,12].

The 5/6 nephrectomy model is a model of renal interstitial fibrosis. We observed different groups of rats for pathological changes in renal function. The levels of BW and blood lipids of the rats in different groups were increased slightly compared with the baseline values, although there were no significant differences between the four groups. Therefore, it's believed that blood lipids have no effect on renal function and renal pathology. The levels of Scr in rats of the group treated with FB (LDCNSN) cells decreased significantly compared with the rats in the group that received FB (LXSN) cells and the operation group, indicating that DCN improved renal function in rats with renal failure. The histopathological examination showed that DCN alleviated the degree of renal interstitial fibrosis in rats and delayed the progression of renal failure.

The expression of DCN and TGF-β1 in renal tissue of rats from Groups B, C, and D, was increased to different degrees compared with those in renal tissue of rats from sham-operated group (Group A). The expression of DCN increased significantly in Group D compared with Group B and C. This was related to the expression of DCN in renal tissue by DCN-expressing fibroblasts. The increase of TGF-β1 is closely associated with the progression of loss of renal function. The 5/6 nephrectomy model is characterized by the increased TGF-β1 expression in renal tissue[13,14]. We found that there were no significant differences in TGF-β1 expression between Group B, C, and D, indicating that the different treatments given to rats with chronic renal failure caused no significant changes in TGF-β1 expression. Further experiments using immunohistochemistry are required to determine whether it is associated with the amount TGF-β1 protein or TGF-β1 activity. Therefore, the balance of DCN/TGF-β1 in renal tissue of rats with renal failure is changed toward DCN after treatment with DCN. Large amounts of DCN can neutralize TGF-β1 and inhibit pathological changes to the kidney, which in turn improve renal function[15]. Previous researches[16,17] have shown that the expression of DCN in renal tissue in rodent models of diabetic nephropathy and obstructive nephropathy was not decreased but increased. Therefore, it is thought that the expression of DCN during the progression of renal diseases is insufficient and cannot neutralize TGF-β1 activity.

Exogenous DCN or DCN gene therapy can prevent the accumulation of extracellular matrix resulting from TGF-β1 activity[18]. Using the anti-Thyl-1 nephritis model, Lishmanov et al[19] demonstrated that increases in TGF-β1 can cause increased synthesis of DCN. They proposed that DCN may serve as a growth inhibitor that inhibits the production of mesangial matrix components. Hence, DCN can serve as a critical factor that controls excessive production of TGF-β1 and can potentially be used for the clinical treatment of glomerular diseases[20]. Ghosh et al[21] showed that injections of pharmacological doses of DCN (450 μg) in the anti-Thyl-1 nephritis model prevented excessive accumulation of matrix components and the progression of proteinuria. No effect was observed at 1-2 d after application, while extracellular matrix components, such as fibronectin (FN) and fibronectin extra domain-a (FN-EDA) decreased significantly at 4-6 d after application[22]. Researches[23,24] have also shown that diseased glomeruli appeared mostly normal and DCN had no toxic effect on the patients treated with DCN. Several studies have attempted to demonstrate the diagnostic value of DCN by applying gene therapy. DCN cDNA was injected into host skeletal muscle via liposomes to transfect skeletal muscle cells. DCN protein was synthesized in myocytes and secreted into blood, and acts on the kidneys via blood flow. The expression of FN-EDA and type-1 collagen within glomeruli decreased significantly after treatment. The levels of proteinuria also decreased significantly. There were no significant differences in functions of glomeruli between the treatment group and normal control group at day 14[25,26].

Conclusions

We showed that in rats DCN can markedly alleviate fibrosis and delay the progression of renal failure.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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