1,25-Dihydroxyvitamin D₃ Ameliorates Podocytopenia in Rats with Adriamycin-induced Nephropathy

Min-shu Zou¹, Jian Yu¹, Jian-hua Zhou², Guo-ming Nie¹, Dong-sheng Ding¹, Li-man Luo¹, Hong-tao Xu¹ and Wei-sun He³

Abstract

Objective To investigate the role of α3β1 integrin and α/β-dystroglycan in protective effects of 1,25(OH)₂D₃ on podocytes in rats with adriamycin-induced nephropathy.

Methods Sprague-Dawley rats were randomly divided into three groups: control group (NC), nephropathy group (NE), and nephropathy+1,25(OH)₂D₃ group (ND). Rats in NE and ND group were injected intravenously with adriamycin (0.1 mg/10 g body weight) to induce nephropathy, and those in ND group were then subcutaneously treated with 1,25(OH)₂D₃ for 8 weeks. Urinary protein level, number of urine podocytes, foot process width and glomerulosclerotic index were determined. Nephrin and podocin mRNA and protein expressions were determined by RT-PCR and western blot, respectively. Podocyte density and expressions of α3β1 integrin and α/β-dystroglycan (DG) were analyzed by immunohistochemistry and western blot, respectively.

Results The increase in proteinuria, podocyturia and width of foot process in NE group were ameliorated after treatment with 1,25(OH)₂D₃ for 8 weeks. The glomerulosclerotic index was significantly decreased in ND group when compared with NE group. The podocyte density in ND group (10.3 ± 1.64 cells/glomerulus) was significantly higher than that in NE group (8.43 ± 1.75 cells/glomerulus) (p=0.008). 1,25(OH)₂D₃ treatment could significantly up-regulate the mRNA and protein expressions of nephrin and podocin, and the protein expressions of α3β1 integrin and α/β-DG.

Conclusion The expressions of nephrin, podocin, α3β1 integrin and α/β-DG were decreased in rats with nephropathy. However, 1,25(OH)₂D₃ treatment could significantly up-regulate the expressions of nephrin, podocin, α3β1 integrin and α/β-DG proteins which might suppress podocyte detachment and podocytopenia.

Key words: vitamin D, podocyte, kidney sclerosis, integrin, dystroglycan

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Introduction

Increasing experimental and clinical evidence has demonstrated that the podocyte number is a critical determinant in the development of glomerulosclerosis (1), and podocytopenia may lead to massive proteinuria and ultimate renal scarring (2). The causes of podocytopenia include apoptosis, detachment, and inability or lack of podocytes to proliferate (3). Nephrin and podocin are both component proteins of the slit diaphragm complex with a high degree of podocyte specificity (4). Nephrin is a protein necessary for the proper functioning of the renal filtration barrier and mainly prevent the plasma proteins (especially the albumin) from filtration. Nephrin is a critical protein expressed on the podocytes to impede the albumin filtration. Podocin localizes to the slit diaphragm of podocytes and interacts with the transmembrane adhesion protein nephrin. This interaction is required for efficient signaling through nephrin and its associated proteins (5). α3β1 integrin and α/β-dystroglycan (α/β-

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demonstrated that 1,25(OH)2D3 could significantly reduce proteinuria in rats with adriamycin-induced nephropathy. Our results also indicated that podocyte is a potentially important target for the renoprotective effects of 1,25(OH)2D3. These results clearly identified podocyte hypertrophy in subtotally nephrectomized (SNX) rats (10). These results clearly identify podocyte as a potentially important target for the renoprotective effects of 1,25(OH)2D3 on podocytopenia and nephropathy. However, the mechanism of 1,25(OH)2D3 decreasing podocyte detachment remains poorly understood. The present study aimed to investigate the potential mechanism underlying the protective effects of 1,25(OH)2D3 on podocytopenia in rats with adriamycin-induced nephropathy. Our results demonstrated that 1,25(OH)2D3 could significantly reduce proteinuria, and alleviate podocyte loss and glomerulosclerosis of adriamycin-treated rats and these protective effects may be related to the up-regulation of nephrin, podocin, α/β-integrin and α/β-DG.

Materials and Methods

Establishment of experimental nephropathy model

Seventy-five 7-week-old male Sprague-Dawley (SD) rats weighing 180-200 g (Experimental Animal Center of Wuhan General Hospital) were housed in single cages at constant room temperature (20°C) and humidity (50%). These rats were given ad libitum access to food and water. A nephropathy model was induced according to previously described (11). In brief, rats were intravenously injected with a single dose of adriamycin (0.1 mg/10 g body weight). Proteinuria and biochemical abnormalities appear within 10 days following the injection of ADR and reach relatively stable levels at 4–5 weeks after ADR administration. Four weeks after injection, when proteinuria was stable, rats were randomly assigned into two groups: nephropathy group (NE, n=12), and nephropathy+1,25(OH)2D3 (ND, n=12), with matched body weight and urinary protein-to-creatinine ratio. Rats in NG group received 1,25(OH)2D3 treatment (3 ng/100 g body weight daily) through a subcutaneous osmotic mini-pump (10). Normal rats served as control group (NC, n=10). Rats in NE group and NC group were given equivalent volume of propylene glycol through a subcutaneous osmotic mini-pump. Four and eight weeks later, these rats were placed in metabolic cages and 24-hr urine samples were collected followed by determination of 24-hr urinary protein and podocyte number, and blood was obtained to detect the levels of urea nitrogen (BUN), creatinine (Cr), Cystatin C (Cys C), calcium (Ca), phosphorus (P), albumin (A) and parathyroid hormone (PTH). 24-h UP was measured using the Biuret method, and Cys C level detected with a rat ELISA kit (Bode Biology-Technology Co., Ltd., Shanghai, China). Levels of BUN, Cr, Ca, P and A were analysis with an automatic biochemistry analyzer (Hitachi 7170A, Tokyo, Japan). Serum PTH was measured with a rat PTH immunoradiometric assay (Nichols Institute Diagnostics, San Clemente, CA, USA). After 8 weeks of 1,25(OH)2D3 treatment, all rats were sacrificed for histological analysis and detection of expressions of α/β-integrin and α/β-DG. The whole study was approved by the Animal Care Committee (Wuhan General Hospital).

Immunofluorescence staining for quantification of urinary podocytes

Urinary podocytes (UPCs) were measured as previously described (12). The podocyte-specific marker-podocalyxin (PCX) was detected in urinary sediment by immunofluorescence to evaluate the excretion of UPCs (urinary PCX-positive cells were identified as UPCs). In brief, the 24-hour urine was collected with metabolic cages (10 mL per rat) and centrifuged at 700 g for 5 minutes. The sediment was washed, re-suspended in phosphate-buffered saline (PBS), cyto-centrifuged for 5 minutes at 700 g onto poly-L-lysine-coated microscope slides, and air-dried for at least 30 minutes. The slides were then fixed for 5 minutes in acetone at 4°C. After being washed with PBS, the slides were incubated with 20 μL mouse anti-human podocalyxin monoclonal antibody (1 : 200; R & D Systems, Minneapolis, MN, USA) for 60 minutes. After another washing, the slides were incubated with fluorescein isothiocyanate (FITC) conjugated, affinity-purified anti-mouse IgG (ZhongShan, Inc.; Beijing, China) antibody. The slides were then washed and examined under immunofluorescence microscope. The cell nuclei were counterstained with ethidium bromide before mounting. Twenty high-power fields were randomly selected and PCX-positive cells in urinary sediment were observed under a fluorescence microscope. The number of urinary podocytes was presented as average PCX-positive cell number per field.

Quantitation of glomerular fibrosis

To evaluate the histopathology of kidneys, 3-μm sections
were obtained and stained with periodic acid-Schiff (PAS) reagent. The glomerulosclerotic index (GSI) was graded based on the severity of glomerular damage, including mesangial matrix expansion, hyalinosis with focal adhesion, capillary dilatation, glomerular tuft occlusion, and sclerosis, using a semi-quantitative scoring method: grade 0, normal glomeruli; grade 1, injured glomerular area up to 25%; grade 2, injured glomerular area 25 to 50%; grade 3, injured glomerular area 50 to 75%; grade 4, injured glomerular area 75 to 100%. Twenty glomeruli per section were assessed with the observer masked to the treatment groups and GSI was calculated as follows (13):

\[
\text{GSI} = \frac{(1 \times n_0) + (2 \times n_1) + (3 \times n_2) + (4 \times n_3)}{(n_0 + n_1 + n_2 + n_3 + n_4)}
\]

where \(n_x\) is the number of glomeruli in each grade.

**Immunohistochemistry for quantification of podocyte number per glomerulus cross-section**

For histological analysis of glomeruli, tissues were formalin-fixed, paraffin-embedded and sectioned followed by staining with periodic-acid Schiff (PAS) reagent. Then, 3-μm sections were stained with Wilms tumor antigen-1 (WT-1) antibody (1:50; rabbit polyclonal) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Thirty glomeruli in cross section were randomly selected and the number of WT-1 positive cells was counted (14). Results were presented as average podocyte number per glomerulus cross-section.

**Measurement of foot process width by transmission electron microscopy**

Part of renal cortex was fixed in 1.5% glutaraldehyde and 1% paraformaldehyde, dehydrated, and embedded in Spurr resin. A maximum diameter and its perpendicular diameter were obtained under a light microscope (×200) followed by averaging and then the size of glomeruli was determined. Glomeruli with similar volumes were selected for the detection of FPW. Ultrathin sections were prepared and stained with lead citrate for transmission electron microscopy. Fifteen micrographs at a magnification of ×15,000 were randomly selected from each specimen. The length of peripheral GBM was measured and the number of foot processes on GBM was counted. The mean foot process width (FPW) was calculated as follows: FPW=(π/4)×{(ΣGBM length)/(Σfoot process)}; where ΣGBM length is the total length of GBM in one glomerulus, Σfoot process is the total number of foot processes, and π/4, a correction factor, serves to correct the random orientation under which foot processes are sectioned.

**Expression of nephrin and podocin mRNA and protein by RT-PCR and Western blot assay**

After removal of kidney capsule, the outer cortex was minced in 1- to 2-mm fragments, passed through consecutive 80- and 120-mesh sieves, and recovered from the 200-mesh sieve. Total RNA was extracted from glomeruli with Trizol (MRC Inc., Cincinnati, OH, USA) according to the manufacturer’s instructions. Then, 2 μg of RNA were reversely transcribed into cDNA and 2 μL of cDNA were used as templates for amplification of nephrin, podocin and GAPDH by PCR. The primers were synthesized by Ruijing Biotech, Co., Ltd. (Zhejiang, China) (1) nephrin: forward: 5’ TACACACAGATTCCACG3’, reverse: 5’ GGGCTGGCC-TGTATGTATT3’, and the anticipated length of products was 229 bp; (2) podocin: forward: 5’ TCAAGCCTCTGGATTAG3’, reverse: 5’ CTCATCTCCTGTGGCAT3’, and the anticipated length of products was 392 bp; (3) GAPDH: forward: 5’ CAGTGCACGCTGTTGGCAT3’, reverse: 5’ AGGGGCATCCAGTCTTCTC3’, and the anticipated length of products was 596 bp. The PCR conditions included pre-denaturation at 94°C for 2 minute and 30 cycles of denaturation at 94°C for 30 second, annealing at 55°C (nephrin) or 52°C (podocin) or 53°C (GAPDH) for 30 second and extension at 72°C for 40 second, and a final extension at 72°C for 5 minute. The amplified products were subjected to 1.0% agarose gel electrophoresis and the bands were captured followed by analysis of the optical density. The mRNA expressions of nephrin and podocin were normalized by that of GAPDH.

The protein expression of nephrin and podocin were determined by western blot. Briefly, 75 μg of total proteins from cell-free supernatants were subjected to 8% SDS-PAGE. Proteins were then transferred electrophoretically onto nitrocellulose membranes that were incubated with anti-nephrin and anti-podocin polyclonal antibodies. The expressions of nephrin and podocin were normalized by that of GAPDH.

The protein expression of nephrin and podocin were determined by western blot. Briefly, 75 μg of total proteins from cell-free supernatants were subjected to 8% SDS-PAGE. Proteins were then transferred electrophoretically onto nitrocellulose membranes that were incubated with anti-nephrin and anti-podocin polyclonal antibodies. The expressions of nephrin and podocin were normalized by that of GAPDH as an internal reference. Experiment was performed three times.

**Expression of α3β1 integrin and α/β-DG protein by Western blot assay and indirect immunofluorescence**

The separation of glomeruli was performed as abovementioned. After centrifugation, supernatant was abandoned and glomeruli were obtained. Total proteins of glomeruli were extracted utilizing RIPA buffer. Then, 75 μg of total proteins were subjected to SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes for 2 hour. After being blocked with 5% low-fat milk, the membranes were incubated with anti-αβ3. (Beijing Leagene Biotech. Co., Ltd.) integrin and α/β-DG antibodies (1:100). Subsequently, the membranes were rinsed with Tris-buffer containing 0.02% Tween-20 and incubated with horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG antibodies (1:4,000; Santa Cruz Biotechnology, Inc). Color development was performed with an enhanced chemiluminescence reagent (Santa Cruz Biotechnology, Inc), and the protein bands were quantitated. Expressions of αβ3 integrin and α/β-DG were normalized to that of β-actin (300 kDa) as an internal reference. The optical density (OD) of bands of αβ3 integrin and α/β-DG protein was obtained with a gel electrophoresis image analysis system. Experiment was per-
Table 1. Levels of 24-h UP and UPCs in Different Groups

<table>
<thead>
<tr>
<th></th>
<th>NC (n=10)</th>
<th>NE (n=12)</th>
<th>ND (n=12)</th>
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<tbody>
<tr>
<td>24h UP (mg)</td>
<td></td>
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<tr>
<td>8 wk</td>
<td>6.06 ± 1.94</td>
<td>44.1 ± 13.2*</td>
<td>33.4 ± 7.19**</td>
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<tr>
<td>12 wk</td>
<td>5.63 ± 2.16</td>
<td>58.9 ± 17.4*</td>
<td>39.6 ± 10.5**</td>
</tr>
<tr>
<td>UPC (podocytes/HP)</td>
<td>0.20 ± 0.42</td>
<td>1.70 ± 0.60*</td>
<td>1.30 ± 0.59*</td>
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<tr>
<td>8 wk</td>
<td>0.30 ± 0.67</td>
<td>2.62 ± 0.72*</td>
<td>1.13 ± 0.39**</td>
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NC, normal control group; NE, nephropathy group; ND, nephropathy + 1,25(OH)₂D₃ group; 24-h UP, 24 h urinary protein; UPCs, urinary podocytes.

Data were expressed as means ± SD.

*p < 0.01, **p < 0.05 v.s. NC group at the same time point, *p < 0.01, **p < 0.05 v.s. NE group at the same time point.

Table 2. Blood Biochemical Indicators in Different Groups

<table>
<thead>
<tr>
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<th>NC (n=10)</th>
<th>NE (n=12)</th>
<th>ND (n=12)</th>
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<tbody>
<tr>
<td>BUN (mmol/L)</td>
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<tr>
<td>8 wk</td>
<td>4.41 ± 1.14</td>
<td>6.39 ± 1.31*</td>
<td>6.08 ± 1.26*</td>
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<tr>
<td>12 wk</td>
<td>4.38 ± 1.29</td>
<td>7.85 ± 1.97*</td>
<td>6.22 ± 1.10*</td>
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<td>Cr (μmol/L)</td>
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<tr>
<td>8 wk</td>
<td>73.7 ± 13.5</td>
<td>116 ± 28.7*</td>
<td>104 ± 25.5**</td>
</tr>
<tr>
<td>12 wk</td>
<td>76.6 ± 19.8</td>
<td>179 ± 53.0</td>
<td>109 ± 24.7**</td>
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<tr>
<td>Cys C(mg/L)</td>
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<tr>
<td>8 wk</td>
<td>0.71 ± 0.21</td>
<td>1.18 ± 0.34*</td>
<td>0.85 ± 0.42**</td>
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<tr>
<td>12 wk</td>
<td>0.68 ± 0.23</td>
<td>1.93 ± 0.50*</td>
<td>0.97 ± 0.32**</td>
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<tr>
<td>Ca (mmol/L)</td>
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<tr>
<td>8 wk</td>
<td>4.20 ± 0.52</td>
<td>3.63 ± 0.66**</td>
<td>4.53 ± 0.67**</td>
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<tr>
<td>12 wk</td>
<td>4.12 ± 0.48</td>
<td>3.31 ± 0.67*</td>
<td>4.34 ± 0.54*</td>
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<tr>
<td>P (mmol/L)</td>
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<td></td>
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<tr>
<td>8 wk</td>
<td>1.32 ± 0.19</td>
<td>1.52 ± 0.29</td>
<td>1.39 ± 0.27</td>
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<tr>
<td>12 wk</td>
<td>1.38 ± 0.18</td>
<td>2.03 ± 0.41*</td>
<td>1.41 ± 0.30*</td>
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<tr>
<td>A(g/L)</td>
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<tr>
<td>8 wk</td>
<td>36.3 ± 3.46</td>
<td>25.5 ± 3.83*</td>
<td>29.1 ± 2.75**</td>
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<tr>
<td>12 wk</td>
<td>37.4 ± 3.27</td>
<td>19.8 ± 3.59*</td>
<td>24.9 ± 3.68*</td>
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<tr>
<td>PTH(pg/mL)</td>
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<tr>
<td>8wk</td>
<td>31.3 ± 2.65</td>
<td>57.4 ± 4.55*</td>
<td>47.8 ± 4.15</td>
</tr>
<tr>
<td>12 wk</td>
<td>33.5 ± 2.51</td>
<td>60.8 ± 4.12*</td>
<td>50.5 ± 3.75*</td>
</tr>
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</table>

NC, control group; NE, nephropathy group; ND, nephropathy + 1,25(OH)₂D₃ group; BUN, blood urea nitrogen; Cr, creatinine; Cys C, cystatin C; Ca, calcium; P, phosphorus; A, albumin; PTH, parathyroid hormone.

Data were expressed as means ± SD.

*p < 0.01, **p < 0.05 v.s. NC group at the same time point, *p < 0.01, **p < 0.05 v.s. NE group at the same time point.

Kidney tissues were cut into 3 μm sections and stored at -20°C for use. Rabbit anti-α3β1 integrin and anti-α/β-DG primary antibodies were purchased from Biofriendship Inc (Beijing, China). Bio-Rad Radiance 2100 TM confocal system (Hercules, CA) was used. Ten photographs of glomeruli from each rat were randomly obtained with a digital camera at a magnification of ×400.

Statistical analysis

Data were expressed as means ± SD. Statistical analysis was performed with SPSS version 11.0 statistical package. Multiple comparisons were performed using the Kruskal-Wallis non-parametric test followed by Mann-Whitney U test. Correlations between different variables were assessed with the Spearman correlation analysis. A value of p<0.05 was considered statistically significant.

**Results**

Effect of 1,25(OH)₂D₃ treatment on 24-h UP, UPCs and serum biochemistry indicators

After 8 weeks of treatment, levels of 24-h UP, UPCs, BUN, Cr, Cys C, and PTH in NE group were significantly elevated (p<0.01), Ca level was reduced (p<0.05), and the A level was markedly reduced (p<0.01) when compared with NC group. The decrease in levels of 24-h UP, Cys C, and A and increase in Ca and PTH levels in ND group were significantly attenuated when compared with the NE group. After 12 weeks of treatment, the levels of 24-h UP, UPCs, BUN, Cr, Cys C, P, and PTH in rats of NE group were significantly higher than those in control group. However, the Ca and A levels in NE group were significantly lower than those in NC group and ND group. In general, the protective effects of 1,25(OH)₂D₃ were more prominent at week 12 as compared with those at week 8. Results are shown in Table 1, 2.
Effect of 1,25(OH)2D3 treatment on renal histomorphology

Histological examination by light microscopy revealed no evident glomerular injury in NC group. Accumulation of ECM and segmented sclerosis were observed in a fraction of glomeruli of NE group and the capillary tuft volume per glomerulus was significantly decreased when compared with NC group. GSI was 0.17 ± 0.08 in normal rats, 1.94 ± 0.46 in nephropathy rats, and 1.31 ± 0.33 in 1,25(OH)2D3-treated nephropathy rats. GSI was significantly decreased in ND group when compared with NE group. Electron microscopy showed that FPW was 358 ± 13.7 nm in NC group, 2,073 ± 562.8 nm in NE group, 863 ± 68.5 nm in ND group. The increase in FPW were ameliorated after treatment with 1,25(OH)2D3 (Fig. 1, 2).

1,25(OH)2D3 treatment decreases podocyte loss in nephropathy rats

The average number of podocytes per glomerulus, which was determined by counting cells positive for WT-1, was 12.3 ± 1.47 cells/glomerulus in control rats, 8.43 ± 1.75 cells/glomerulus in nephropathy rats and 10.3 ± 1.64 cells/glomerulus in 1,25(OH)2D3-treated nephropathy rats. The average number of podocytes per glomerulus was significantly decreased in nephropathy rats when compared with 1,25(OH)2D3-treated nephropathy rats (p=0.008) (Fig. 3).

1,25(OH)2D3 treatment up-regulates the mRNA and protein expressions of nephrin and podocin in the nephropathy rats

The mRNA expressions of nephrin and podocin were 0.68 ± 0.13 and 0.82 ± 0.09, respectively, in the control group, 0.45 ± 0.09 and 0.61 ± 0.10 in nephropathy rats, and 0.62 ± 0.10 and 0.73 ± 0.10 in 1,25(OH)2D3-treated nephropathy rats. The protein expression of nephrin and podocin were 0.45 ± 0.12 and 0.48 ± 0.13 in control group, 0.22 ± 0.08 and 0.24 ± 0.05 in nephropathy rats, and 0.36 ± 0.10 and 0.35 ± 0.11, respectively in 1,25(OH)2D3-treated nephropathy rats. Compared with the NC group, the mRNA and protein expressions of nephrin and podocin were significantly decreased in the NE group. However, 1,25(OH)2D3 treatment could significantly up-regulate the expressions of nephrin and podocin (Fig. 4).
1,25(OH)₂D₃ treatment up-regulates protein expression of αβ₁ integrin and αβ-DG in nephropathy rats

Western blot The protein expressions of αβ₁ integrin and α-DG were 0.83 ± 0.13 and 0.87 ± 0.16, respectively, in the control group, 0.49 ± 0.11 and 0.60 ± 0.11 in nephropathy rats, and 0.64 ± 0.13 and 0.73 ± 0.12 in 1,25(OH)₂D₃-treated nephropathy rats. The expression of β-DG was 0.65 ± 0.16 in control group, 0.44 ± 0.12 in nephropathy rats, and 0.54 ± 0.13 in 1,25(OH)₂D₃-treated nephropathy rats. Compared with NC group, the protein expressions of αβ₁ integrin and αβ-DG were significantly decreased in NE group. 1,25(OH)₂D₃ treatment could significantly up-regulate the expressions of αβ₁ integrin and αβ-DG. Results are shown in Fig. 5.

Immunofluorescence staining and distribution of αβ₁ integrin and αβ-DG In the control group, fine and linear-like distribution of αβ₁ integrin was detected along the glomerular capillary loop; linear-like distribution of αβ-DG was noted but more dispersed than that of αβ₁ integrin. In nephropathy rats without treatment, distribution of αβ₁ integrin and αβ-DG was discontinuous throughout the glomeruli and coarse granules positive for αβ₁ integrin and αβ-DG were observed in some glomerular capillary walls. Treatment with 1,25(OH)₂D₃, significantly, although not completely, restored αβ₁ integrin and αβ-DG staining and the distribution was similar to that in the control group (Fig. 6).

Correlation analysis There was a significantly positive correlation between UPCs and 24-h UP (r=0.42, p<0.01). There was a negative correlation between UPCs and protein expressions of nephrin, podocin, αβ₁ integrin, α-DG, and β-DG (r=-0.65, -0.47, -0.55, -0.51, and -0.45, respectively; p<0.01).

Discussion Proteinuria is an important indicator of compromised glomerular filtration barrier. Podocytes are the final barrier in glomerular filtration membrane. After stimulation with numerous factors, the podocytes can detach from the GBM and then be excreted along with emiction. The damage to podocytes is one of critical mechanisms in the occurrence of proteinuria and glomerulosclerosis. Nephrin and podocin locate in the slit diaphragm and are the main proteins impeding protein infiltration. Abnormal signal transductions between nephrin and podocin may compromise the function of podocytes and result in proteinuria. Abnormal nephrin ex-
Figure 4. mRNA and protein expressions of nephrin and podocin in the glomeruli. (A) 1,4,7: NC group; 2,5,8: NE group; 3,6,9: ND group. The mRNA expressions of nephrin and podocin (A and B) had similar pattern to expressions of both proteins (C and D). When compared with NC group, the mRNA and protein expressions of nephrin and podocin in the NE group were markedly decreased (p<0.01). Furthermore, when compared with the NE group, the mRNA expressions of nephrin and podocin were increased by 37.8% and 19.7%, respectively (p<0.01) and the protein expressions were elevated by 63.6% and 45.8%, respectively (p<0.01) in the ND group. Data were expressed as means ± SD. *p<0.01, **p<0.05 v.s. control group; * p<0.01, ** p<0.05 v.s. NE group.

Figure 5. Expressions of αβ1 integrin, α-DG, and β-DG in different groups. Compared with control group, the protein expressions of αβ1 integrin and α/β-DG were down-regulated in nephropathy rats. Compared with nephropathy rats, the decreased expressions of αβ1 integrin and α/β-DG were alleviated after 1,25(OH)2D3 treatment. Data were expressed as means ± SD. *p<0.01, **p<0.05 v.s. control group; * p<0.01, ** p<0.05 v.s. NE group.
expression induces proteinuria, and the recovery of nephrin expression accompanies reductions in proteinuria. Yamauchi et al (15) demonstrated that active vitamin D directly binds VDR in podocytes and increases the transcription of nephrin mRNA in cultured murine podocytes. Attachment of podocytes to the GBM is proposed to be mediated by α3β1 integrin complex and DG (16). The α3β1 integrin complex is a major extracellular matrix receptor complex expressed in podocytes, and plays a critical role in the adherence of foot processes to the GBM (17). α3 integrin-deficient mice are not able to produce intact foot processes (18). Anti-β1 integrin antibodies lead to an increase of albumin permeability in isolated glomeruli (19). Reduced expression of α3β1 integrin in podocytes has been demonstrated in both humans with FSGS (6) and in diabetic (20) and PAN model rats (21). Podocyte foot processes are anchored to the GBM via α3β1 integrin (22) and α/β-DG (23). Our study revealed that down-regulation of α3β1 integrin and α/β-DG expression disturbed the interaction between α3β1 integrin and/or DG and the GBM, resulting in podocyte detachment and loss. Podocyte loss results in a localized “bare” or denuded GBM and podocytopenia in glomeruli, which cause glomerulosclerosis, proteinuria, and renal dysfunction (24). There was a significantly positive correlation between UPCs and 24-h UP, which illustrates that podocyte detachment plays an important role in the occurrence and development of proteinuria. Herein, we also demonstrated that 1,25(OH)2D3 treatment in nephropathy rats could up-regulate the expressions of nephrin and podocin, α3β1 integrin and α/β-DG, and decrease podocyte loss and FPW, which may lead to improvement in podocytopenia, proteinuria and a decrease in the urinary podocyte number.

It has been shown that proteinuria is associated with foot process effacement (25). Moreover, podocyturia is a more sensitive indicator in assessing the degree of glomerular damage than proteinuria in SD rats (26). Chen et al (6) showed that decreased expression of α3β1 integrin in podocytes was closely related to podocyte depletion, glomerular sclerosis, and daily protein loss in patients with primary FSGS and chronic PAN-treated rats. Down-regulation of α3β1 integrin expression is per se sufficient to reduce podocyte adhesion (27). α-DG was found to play important roles in maintaining unique architecture of podocytes by binding to the GBM, and in maintaining the integrity of filtration slit through negatively charged sialic acid residues (28). Pagtalunan et al (29) also showed that podocytopenia correlated with microalbuminuria, renal failure, and development of glomerulosclerosis in patients with type II diabetes. Podocytes are known to express vitamin D receptors. Recent reports also indicate that 1,25(OH)2D3 has direct effects on po-
podocytes. 1,25(OH)D$_3$ could decrease podocyte loss and inhibit podocyte hypertrophy in SNX rats (10). 1,25(OH)D$_3$ could reduce proteinuria in active Thy1-nephritis rats (30) and inhibit progressive glomerulosclerosis and decrease albuminuria in SNX rats (31). Li et al (32) recently noted that 1,25(OH)D$_3$ was a negative endocrine regulator of the renin-angiotensin system (RAS). Matsui et al (33) demonstrated that impairment of the vitamin D system played a role in proteinuria after podocyte injury. These findings suggest that 1,25(OH)D$_3$ may be a clinically useful agent for preventing podocyteopenia and progression of glomerulosclerosis. Our results were partly in accordance with the above-mentioned studies. Regele et al (7) demonstrated that α/β-DG expression in glomeruli was reduced in minimal change nephropathy but not in FSGS. However, our results showed that α/β-DG expression was markedly decreased in nephropathy rats. The disparity between our study and others may result from different time points of observation and different methods for quantitation. In the podocytes, proteins of Smads family play an important role in the signal transduction from cell membrane to nucleus. TGF-β1 can activate smad2/3 mediating podocyte apoptosis. In the podocytes, proteins of Smads family play an important role in the signal transduction from cell membrane to nucleus. TGF-β1 can activate smad2/3 mediating podocyte apoptosis. BMP-7 can activate Smad l/5/8 protecting podocytes. Xiao et al (34) reported the protective effects of 1,25(OH)D$_3$ on podocytes were mediated by TGF-β1/BMP-7. Therefore, 1,25(OH)D$_3$ may confer protective effects on the podocytes through suppressing TGF-β1/smads2/3 pathway and activating BMP-7/Smad l/5/8 pathway. In our study, serum Ca level was significantly elevated and P, and PTH levels decreased in ND group when compared with NE group, but the levels of Ca and P were not different from those in NC group at week 12. Decreased serum Ca level can promote the excretion of PTH, and elevated PTH level are associated with progressive hyperparathyroidism. 1,25(OH)D$_3$ not only promotes the absorption of Ca by the intestine but also maintains the Ca homeostasis. It can be used to prevent and treat hyperparathyroidism. Furthermore, no potential adverse events were noted during the treatment. However, our study only investigated the renal histomorphology after 8 weeks of treatment with 1,25(OH)D$_3$, and the long-term effects of 1,25(OH)D$_3$ on podocyte density and the expressions of adhesion molecules should be further explored.

1,25(OH)D$_3$ is widely used in regulating calcium and phosphorus metabolism in patients with chronic kidney disease. However, the cellular mechanisms by which 1,25(OH)D$_3$ prevents podocyteopenia have not been clarified to date. Thus, our findings, together with the other above-mentioned in vitro studies, suggest that 1,25(OH)D$_3$ can prevent podocyte detachment and podocyteopenia in nephropathy rats at least partially through up-regulating expressions of nephrin and podocin, α/β integrin and α/β-DG. However, more studies are necessary to elucidate the exact mechanisms of the renoprotective effects of 1,25(OH)D$_3$.

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References