1,25-Dihydroxyvitamin D₃ decreases podocyte loss and podocyte hypertrophy in the subtotally nephrectomized rat

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Departments of ¹Pathology and ²Internal Medicine, ³Institute of Biomedicine, University of Erlangen-Nuernberg, D-91054 Erlangen; and Departments of ³Pathology and ²Internal Medicine, University of Heidelberg, 69115 Heidelberg, Germany

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Kuhlmann, Alexander, Christian S. Haas, Marie-Luise Gross, Udo Reulbach, Marc Holzinger, Ute Schwarz, Eberhard Ritz, and Kerstin Amann. 1,25-Dihydroxyvitamin D₃ decreases podocyte loss and podocyte hypertrophy in the subtotally nephrectomized rat. Am J Physiol Renal Physiol 286: F526–F533, 2004. First published November 4, 2003; 10.1152/ajprenal.00316.2003.—1,25(OH)₂D₃ has antiapoptotic effects and promotes cell differentiation. This consideration has provided the rationale for studies in subtotally nephrectomized rats showing that 1,25(OH)₂D₃ interfered with glomerulosclerosis. The cellular mechanisms involved have remained obscure, however. It was the purpose of the present study to assess glomerular structure and cellular composition in subtotally nephrectomized (SNX) rats treated with nonpharmacological doses of 1,25(OH)₂D₃. Male Sprague-Dawley rats were sham operated (sham) or underwent SNX under general anesthesia and received either solvent or 1,25(OH)₂D₃ (3 ng/kg/day). Blood pressure (BP), serum creatinine, and urinary albumin excretion were significantly higher in SNX than in sham rats. Albuminuria was significantly lower in SNX+1,25(OH)₂D₃ compared with SNX+solvent rats. Mean glomerular tuft volume was significantly higher in SNX+solvent (2.69 ± 0.21 × 10⁶ μm³) than in sham rats (1.44 ± 0.17 and 1.28 ± 0.14 × 10⁶ μm³); it was significantly (P < 0.05) lower in SNX+1,25(OH)₂D₃ (1.81 ± 0.16 × 10⁶ μm³). The main finding was a significantly higher number of podocytes in SNX+1,25(OH)₂D₃ rats (88 ± 9) and sham (98 ± 17) compared with SNX+solvent rats (81 ± 8.7). In parallel, the increase in podocyte volume in SNX+solvent rats was abrogated by treatment with 1,25(OH)₂D₃, and immunohistochemistry revealed less expression of desmin, PCNA, and p27, suggesting less podocyte injury and activation of the cyclin cascade. This study identifies the podocyte as an important target cell for the renoprotective action of 1,25(OH)₂D₃. This notion is suggested by less evidence of podocyte injury, decreased podocytes loss, and abrogation of podocyte hypertrophy, findings that may also explain less pronounced albuminuria and glomerulosclerosis.

visceral glomerular epithelial cell; mesangial cell; glomerular endothelial cell; progression

THE VITAMIN D ENDOCRINE SYSTEM exerts numerous pleiotropic effects unrelated to its traditionally recognized function of regulating plasma calcium levels (7, 31, 53). Previous studies in this laboratory documented that 1,25(OH)₂D₃ treatment

attenuated the development of glomerulosclerosis and progression of albuminuria in subtotally nephrectomized (SNX) rats (45). This observation is in line with several studies assessing the role of active vitamin D in nonimmune (14) and immune-mediated models of injury (2, 24, 33, 35).

These studies found reduced mesangial cell proliferation, glomerulomegaly, and development of glomerulosclerosis by administration of 1,25(OH)₂D₃. Consequently, the action of 1,25(OH)₂D₃ was attributed to prevention of mesangial cell proliferation (14, 45).

There has been increasing recognition of an important role for podocytes in the progression of renal disease (27). It has recently become clear that initial glomerular injury affects the podocyte as an important target cell for progression (21), e.g., in diabetic nephropathy (5, 10), fawn hooded rats (20), or SNX rats (1). This consideration and the fact that podocytes possess receptors for 1,25-dihydroxyvitamin D₃ (49) prompted the present study to assess the action of 1,25(OH)₂D₃ on podocytes in the SNX rat. As signs of podocyte injury, we assessed podocyte number, podocyte volume, and podocyte ultrastructure as well as changes in the pattern of structural molecules and de novo expression of desmin. De novo expression of desmin has recently been recognized as a sensitive early marker of podocyte injury (8, 16). Expression of proliferating cell nuclear antigen (PCNA) in podocytes as postmitotic cells does not necessarily indicate cell proliferation but rather activation of the cyclin cascade (15). We also studied expression of the cyclin-dependent kinase (CDK) inhibitor protein p27 because active vitamin D has been shown to upregulate expression of p27 in numerous cell lines, e.g., retinoic acid-resistant acute promyelocytic leukemia cell lines (U937) (28), squamous carcinoma cell lines of the head and neck (12), and in human breast cancer MCF-7 cells (50). Furthermore, it has recently been shown that subtle injury to postmitotic podocytes leads not only to an abortive upregulation of the cyclin cascade but also to strong upregulation of p21 and p27 (27, 36). The importance of these proteins in preventing podocyte injury is illustrated by the observation in standard models of renal injury of more rapid progression in p21 and p27 knockout mice (18, 26).

MATERIALS AND METHODS

Animals

Twenty-nine male Sprague-Dawley rats (SD; 200 g; Ivanovas, Kisslegg, Germany) were housed in single cages at a constant room temperature prior to the study. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
temperature (20°C) and humidity (25%). The animals received a high-protein (25%) diet with 0.5% NaCl (Altromin, Lage, Germany). After 3 days of adaptation, the animals were randomly allocated to a surgical two-step SNX of moderate intensity or to a sham operation (sham). In the first operation, the right kidney was removed, and 7 days later cortical tissue of the hypertrophied left kidney was resected (see below). Treatment with either solvent (ethanol) or 1,25(OH)2D3 (3 ng/100 g body wt 1-day) was started on the day of the second operation by subcutaneous implantation of osmotic minipumps. After 4 wk the minipumps had to be exchanged under general anesthesia (see below). Blood pressure was measured at the beginning of the experiment and subsequently every second week by tail plethysmography in conscious animals. After 2, 4, and 8 wk, urinary albumin excretion was measured in solvent- and 1,25(OH)2D3-treated sham and SNX animals using a sandwich ELISA system (45). The experiment was terminated using perfusion fixation (see below).

Partial Renal Ablation by Surgical SNX

The right kidney was removed and weighed under ketamine/diazepam anesthesia (100 mg/kg or 2.5 μg/kg, respectively). After 7 days, we partially resected the cortex of the left kidney, corresponding to two-thirds of the weight of the right kidney. Control animals were sham resected by removal of the kidney capsule. Special care was taken to avoid injury to the adrenals (for details, see Ref. 45).

Experimental Protocol

The animals were randomly allocated to the following experimental groups:

1) Sham-operated+solvent (sham+solvent; n = 8)
2) Sham-operated+1,25(OH)2D3 (sham+1,25(OH)2D3; n = 7)
3) SNX+solvent (SNX+solvent; n = 6)
4) SNX+1,25(OH)2D3 (SNX+1,25(OH)2D3; n = 8)

For immunohistochemical investigations (see below), five additional SNX+solvent and five SNX+1,25(OH)2D3 animals were examined.

Perfusion fixation and tissue sampling. Sixteen weeks after the second operation, the experiment was terminated by retrograde perfusion fixation. The abdominal aorta was catheterized under ketamine/diazepam anesthesia (doses as above), and blood samples were taken for determination of serum parameters. The viscera were rinsed with 10% dextran solution containing 0.5 g/l procain hydrochloride for 2 min. Ten seconds later, after aortic perfusion was started, the vena cava was incised to drain the solution containing 0.5 g/l procain hydrochloride for 2 min. Ten seconds after the infusion, the vascular system was perfused with 0.2 M phosphate buffer containing 3% glutaraldehyde (12 min) for morphometric and stereological measurements. For immunohistochemistry, five additional SNX+solvent and SNX+1,25(OH)2D3 animals were perfused using ice-cold NaCl as the perfusion medium. The kidneys were taken out, weighed, and dissected in a plane perpendicular to the interpolar axis, yielding 1-mm-thick slices. Ten small pieces of each kidney (2 × 2 × 1 mm) were selected by area weight, sampling for embedding in Epon-Araldite. Five of the resin blocks were randomly chosen, from which semithin sections (0.8 μm) were prepared and stained with methylene blue and basic fuchsin. For qualitative electron microscopic investigations, several ultrathin sections (0.08 μm) per animal were prepared and stained with uranyl acetate and lead citrate. Sections were then qualitatively investigated using a Zeiss EM 10 (Zeiss, Oberkochen, Germany) at various magnifications.

For immunohistochemical investigations, the kidney was fixed with buffered formalin and embedded in paraffin, and 3-μm-thick sections were cut.

Morphometric and Stereological Measurements

Mean glomerular tuft area. Area (Agl) and volume density (Vgl) of the renal cortex and medulla as well as the number of glomeruli per area (Ngl) were measured using a Zeiss eyepiece (Integrationsplatte II; Zeiss) and the point-counting method (FP = Agl × Vgl) at a magnification of ×400 (1, 54, 55). The total area of the glomerular tuft (AT) was determined as AT = Agl × Vgl × cortex (1.1). The number of glomeruli per volume (Ngl) and the volume density (Vgl) of glomeruli was determined using the formula Ngl = k/β × Nk × Vk × 0.5 with k = 1.1 and β = 1.382 (1). The total number of glomeruli was derived from the total volume of the renal cortex and the number of glomeruli per cortex volume: Ngl/cm3 = Ngl × Vgl/cm³. The mean glomerular tuft volume (Vgl/cm³) was determined according to the volume density equation (Vgl/cm³) = βk × 1.1.5, with β = 1.382 and k = 1.1 (1, 45).

Glomerular capillaries and cells. All investigations of semithin sections were performed in a blinded manner using a magnification of ×1,000 and oil immersion as described (1). In brief, the length density (Lc) of glomerular capillaries was determined according to the standard stereological formula Lc = 2 Qc (with Qc being the number of capillary transsects per area of the glomerular tuft). The total area of glomerular capillaries was derived from Agl × AT. The filtration area (FF) was calculated according to the total area of glomerular capillaries times total the number of glomeruli per kidney. The total number of glomeruli per kidney was determined in paraffin sections as described above.

Glomerular cellularity (podocytes, mesangial, and endothelial cells) was assessed using a 100-point Zeiss eyepiece (Integration-Platte II) and the point-counting method as described by Weibel (54) in at least 20 glomeruli/animal at a magnification of ×1,000. Thus Vv and Nc were determined and the number of cells per volume (Nv): Nv = k/β × Nk × Vk × 0.5 with β = 1.5 for podocytes and β = 1.4 for mesangial and endothelial cells and k =1 (1, 9, 11, 40, 54, 55).

The number of cells per glomerulus (Ngl/cm³) was then derived from the glomerular tuft volume: Ngl/cm³ = Vgl × Vgl/cm³. The mean cell volume (Vc) was determined from volume density, mean glomerular volume, and mean number of cells per glomerulus: Vc = Vgl × Vgl/cm³ × Ngl/cm³.

Immunohistochemical Investigations

Immunohistochemistry was performed in solvent- and in 1,25(OH)2D3-treated SNX animals with the following antibodies: PCNA (dilution 1:500; DAKO, Hamburg, Germany), p27 (rabbit polyclonal antibody; dilution 1:500; Santa Cruz Biotechnology, Heidelberg, Germany), and WTI (rabbit polyclonal; dilution 1:300; Santa

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Wt, g</th>
<th>Wt of Left Kidney (Remnant), g</th>
<th>Systolic Blood Pressure, mmHg</th>
<th>Scu, mmol/l</th>
<th>Sua, mg/dl</th>
<th>Scrac, mg/dl</th>
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<tr>
<td>Sham + solvent (n = 8)</td>
<td>463 ± 30.1</td>
<td>0.74 ± 0.20</td>
<td>113 ± 10.0</td>
<td>2.26 ± 0.12</td>
<td>61.9 ± 13.5</td>
<td>0.95 ± 0.31</td>
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<tr>
<td>Sham + 1,25(OH)2D3 (n = 7)</td>
<td>502 ± 31.2*</td>
<td>0.90 ± 0.27</td>
<td>116 ± 9.8</td>
<td>2.35 ± 0.09</td>
<td>85.6 ± 23.2</td>
<td>1.02 ± 0.26</td>
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<tr>
<td>SNX + solvent (n = 6)</td>
<td>478 ± 39.2</td>
<td>2.20 ± 0.51*</td>
<td>126 ± 8.0†</td>
<td>1.91 ± 0.08*</td>
<td>181 ± 50.7*</td>
<td>2.13 ± 0.90†</td>
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<tr>
<td>SNX + 1,25(OH)2D3 (n = 8)</td>
<td>459 ± 11.6</td>
<td>1.94 ± 0.20</td>
<td>128 ± 13.0†</td>
<td>2.31 ± 0.10*</td>
<td>156 ± 22.2†</td>
<td>1.80 ± 0.29†</td>
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ANOVA

P < 0.05

P < 0.05

P < 0.05

P < 0.05

Values are means ± SD. n, No. of rats; Scu, Sua, and Scrac: serum Ca, urea, and creatinine, respectively; sham, sham-operated control rats; SNX, subtotal nephrectomized rats. Left kidney tissue was perfusion fixed. *P < 0.05 vs. sham + solvent rats. †P < 0.05 vs. sham + 1,25(OH)2D3 rats. ‡P < 0.05 vs. SNX+solvent rats.

Table 1. Animal data
The weight of the left kidney (or remnant kidney) was significantly lower in subtotally nephrectomized (SNX)+solvent- compared with sham-operated (sham) and SNX+1,25(OH)₂D₃-treated Sprague-Dawley rats. *P < 0.05 vs. sham groups. †P < 0.05 vs. SNX+solvent rats.

The urinary albumin excretion rate rose progressively over time in solvent- and 1,25(OH)₂D₃-treated SNX compared with stable values in sham-operated rats (values <0.1 mg/24 h); after 8 wk, it was significantly higher (P < 0.05) in SNX+solvent (5.4 ± 3.89 mg/24 h) than in SNX+1,25(OH)₂D₃ rats (0.95 ± 0.45 mg/24 h). Serum creatinine and urea concentrations were significantly higher in both SNX groups than in sham rats; there was no difference between solvent- and 1,25(OH)₂D₃-treated SNX rats. Serum Ca was significantly lower in SNX+solvent than in the three other groups. The highest serum Ca value in one animal of the SNX+1,25(OH)₂D₃ group was still in the normal range, i.e., 2.56 mmol/l. Serum 1,25(OH)₂D₃ con-

Statistics

All data are expressed as means ± SD. All variables were tested for normal distribution by the Kolmogorov-Smirnov test. One-way ANOVA was performed, followed by the least significant difference test to assess differences between groups. All statistical tests were two tailed, and a significance level of α = 0.05 or less was used.

RESULTS

Description of the Animal Model

Body weight was significantly higher in sham+1,25(OH)₂D₃ than in sham+solvent and SNX+1,25(OH)₂D₃ rats (Table 1). The weight of the left kidney (or remnant kidney) was significantly higher in SNX+solvent than in sham+solvent rats. Systolic blood pressure was comparable in all animals before the operation (115–120 mmHg). At the end of the study, it was significantly higher in SNX than in sham rats but did not differ in SNX+solvent and SNX+1,25(OH)₂D₃ rats, indicating that 1,25(OH)₂D₃ had no effect on blood pressure. The relative heart weight (heart weight-to-body weight ratio) was also significantly higher in SNX+solvent (3.02 ± 0.54 mg/g) compared with both sham-operated groups [sham+solvent: 2.60 ± 0.3 mg/g and sham+1,25(OH)₂D₃: 2.56 ± 0.33 mg/g, respectively]; it was not significantly different in SNX+1,25(OH)₂D₃ compared with SNX+solvent rats. The urinary albumin excretion rate rose progressively over time in solvent- and 1,25(OH)₂D₃-treated SNX compared with stable values in sham-operated rats (values <0.1 mg/24 h); after 8 wk, it was significantly higher (P < 0.05) in SNX+solvent (5.4 ± 3.89 mg/24 h) than in SNX+1,25(OH)₂D₃ rats (0.95 ± 0.45 mg/24 h). Serum creatinine and urea concentrations were significantly higher in both SNX groups than in sham rats; there was no difference between solvent- and 1,25(OH)₂D₃-treated SNX rats. Serum Ca was significantly lower in SNX+solvent than in the three other groups. The highest serum Ca value in one animal of the SNX+1,25(OH)₂D₃ group was still in the normal range, i.e., 2.56 mmol/l. Serum 1,25(OH)₂D₃ con-

Fig. 1. Effect of 1,25(OH)₂D₃ treatment on length density (Lₜ) of glomerular capillaries. Lₜ was significantly lower in subtotally nephrectomized (SNX)+solvent- compared with sham-operated (sham) and SNX+1,25(OH)₂D₃-treated Sprague-Dawley rats. *P < 0.05 vs. sham groups. †P < 0.05 vs. SNX+solvent rats.

Fig. 2. Effect of 1,25(OH)₂D₃ treatment on glomerulosclerosis. Note that mesangial matrix expansion (*), enlargement of podocytes with protein deposition (arrowhead), and adhesion to Bowman’s capsule (arrow) in solvent-treated SNX rats (A) were prevented when SNX rats were treated with 1,25(OH)₂D₃ (B). Magnification: ×1,000.
centration was three times higher ($P < 0.01$) in SNX+1,25(OH)$_2$D$_3$ than in SNX+solvent. Hemoglobin and hematocrit were not significantly different between the experimental groups (data not shown).

**Effect of 1,25(OH)$_2$D$_3$ Treatment on Glomerular Capillary Density**

The length density of glomerular capillaries, i.e., the length of all capillaries per glomerular tuft volume, was significantly lower in SNX+solvent compared with both sham groups. It was higher and no longer different from sham when SNX rats were treated with 1,25(OH)$_2$D$_3$ (Fig. 1). This finding is in agreement with the previous finding (45) of less glomerulosclerosis after treatment with 1,25(OH)$_2$D$_3$ (Fig. 2), because less glomerulosclerosis implies more open capillaries per area of glomerular tuft. Higher glomerular capillary length density was seen in SNX+1,25(OH)$_2$D$_3$ compared with SNX+solvent rats (Fig. 1). The total length of glomerular capillaries was significantly less in SNX+1,25(OH)$_2$D$_3$ than in sham+solute and SNX+solute rats (Table 2). This was due to the fact that glomerular enlargement in SNX+solute rats was partly prevented by 1,25(OH)$_2$D$_3$ treatment (Fig. 2).

**Effect of 1,25(OH)$_2$D$_3$ Treatment on Podocytes**

Mean podocyte number per glomerulus was significantly lower in SNX+solute than in both sham groups indicating loss of podocytes (Table 3). This was not seen when SNX rats were treated with 1,25(OH)$_2$D$_3$. The decrease in podocyte number per glomerulus was paralleled by a significantly ($P < 0.001$) lower number of WT1-positive cells per glomerulus in SNX+solute (4.92 ± 0.11) compared with SNX+1,25(OH)$_2$D$_3$ rats (7.0 ± 0.13). In parallel, mean podocyte volume was significantly higher in SNX+solute than in both sham groups and in SNX+1,25(OH)$_2$D$_3$ rats. This finding indicates that hypertrophy of podocytes occurs after SNX. This was prevented by treatment with 1,25(OH)$_2$D$_3$.

The number of PCNA-positive glomerular cells was significantly ($P < 0.05$) higher in solvent-treated SNX ($2.10 ± 1.64$)  

![Fig. 3. Glomerular desmin staining as a marker of podocyte damage. Desmin staining is much more intensive in solvent-treated SNX (A) compared with 1,25(OH)$_2$D$_3$-treated SNX rats (B), indicating preservation of podocyte morphology after 1,25(OH)$_2$D$_3$ treatment. Magnification: ×1,000.](http://ajprenal.physiology.org/)
compared with SNX+1,25(OH)2D3 rats (0.47 ± 0.29). In contrast, the number of cells, mainly podocytes, staining positive for the cell cycle-inhibitory protein p27 was significantly (P < 0.01) higher in SNX+1,25(OH)2D3 (4.44 ± 1.24) than in SNX+solvent rats (2.6 ± 1.08). The immunohistological staining score of podocytes for desmin, a marker of podocyte damage, was significantly (P < 0.05) lower in SNX+1,25(OH)2D3 (13.4 ± 4.94) than in SNX+solvent rats (18.3 ± 4.22). These findings are illustrated in Fig. 3.

Light microscopy and in particular semithin sections (Fig. 4) documented hypertrophy and degeneration of podocytes with more frequent synechia, i.e., adhesion of the capillary tuft to Bowman’s capsule. Electron microscopic investigations using ultrathin sections (Fig. 5) confirmed podocyte hypertrophy and revealed signs of podocyte degeneration (i.e., cytoplasmic vacuolization, pseudocyst formation, loss or flattening of foot processes, bridging of podocyte cytoplasm) in solvent-treated SNX (Fig. 5, A and E) compared with sham-operated animals (Fig. 5, B and D). As a consequence, denudation of glomerular basement membrane and tuft adhesion to Bowman’s capsule were noted (Fig. 5E). These alterations were not seen in the treated SNX animals (Fig. 5, C and F). In addition, mesangial cell hyperplasia with mesangial matrix deposition was present in solvent-treated SNX (Fig. 5B) compared with sham rats (Fig. 5A), which were prevented by 1,25(OH)2D3 (Fig. 5C). Thus, in summary, glomerular ultrastructure was markedly preserved when SNX rats were treated with 1,25(OH)2D3 (Fig. 5D).

**Effect of 1,25(OH)2D3 on Mesangial and Endothelial Cells**

The number of mesangial cells and endothelial cells per glomerulus was significantly higher in SNX+solvent compared with both sham groups, indicating mesangial and endothelial cell hyperplasia. Both mesangial and endothelial cell hyperplasia were prevented in SNX+1,25(OH)2D3 rats. The mean mesangial and endothelial cell volumes were not significantly different among the four groups [mesangial cell volume: 2,407 ± 248 μm³ in sham+solvent, 2,443 ± 474 μm³ in sham+1,25(OH)2D3, 2,204 ± 560 μm³ in SNX+solvent, and 2,228 ± 209 μm³ in SNX+1,25(OH)2D3; endothelial cell volume: 1,250 ± 120 μm³ in sham+solvent, 1,283 ± 499 μm³ in sham+1,25(OH)2D3, 1,034 ± 250 μm³ in SNX+solvent, and 1,145 ± 266 μm³ in SNX+1,25(OH)2D3]. The changes in glomerular cellularity and capillarization are illustrated in Fig. 4.

**DISCUSSION**

The present study confirms previous findings of this (25, 45) and other laboratories (14, 24) of less glomerular damage in 1,25(OH)2D3–treated rats with renal injury.

These findings are extended by using more sophisticated stereological methodology, e.g., length density of glomerular capillaries as an index of open capillaries per volume of the glomerular tuft. In vivo relevance was also documented by a significant effect of 1,25(OH)2D3 on the urinary albumin excretion rate. Our results confirm previous observations that the mesangial cell number (as a result of glomerular cell proliferation) is less in SNX rats treated with active vitamin D compared with solvent-treated SNX rats.

The novel finding of this study is that the decrease in podocyte number, the increase in podocyte volume, as well as immunohistochemical and ultrastructural indexes of podocyte injury were markedly less pronounced in SNX rats treated with 1,25(OH)2D3 compared with solvent-treated SNX rats. These
findings identify the podocyte as a direct or indirect target for the renoprotective action of 1,25(OH)₂D₃.

The podocyte has recently been recognized as an important mediator in the progression of glomerular damage (21, 27). This is illustrated by clinical observations of podocyte loss in patients with progressive renal diseases, e.g., type 2 (32) or type 1 diabetes (47), and patients with lupus nephritis and focal segmental glomerulosclerosis (51). There is also abundant experimental evidence that the progression of renal injury is associated with progressive podocyte loss (10, 21). It is remarkable that even in the present short-term study, a significant effect of vitamin D on podocyte number could be observed.

Podocytes are known to express vitamin D receptors (48, 49). This observation would be consistent with a direct effect of 1,25(OH)₂D₃ on podocytes. However, we cannot exclude the following alternative explanation: less expansion of glo-
merular volume diminishes the domain that has to be covered by postmitotic podocytes, leading to denudation of the base-ment membrane, formation of glomerular adhesions, and thus to the induction of progressive segmental glomerulosclerosis (9, 21, 41).

It is unclear why the number of podocytes decreases in the SNX animals, and by which mechanisms the decrease of podocyte number was abrogated by 1,25(OH)2D3. In principle, podocyte loss may be caused both by apoptosis (44) and by detachment (27) through interruption of podocyte linkage to the basement membrane via α3β1-integrins (4, 6) and dystroglycan (38, 39), respectively. Evidence for detachment is provided by the observation that vital podocytes can be cul-tured from the urine of animals (19) or patients (13) with progressive renal disease. With respect to apoptosis, a partic-ular comment is appropriate: it is known that activation of the vitamin D receptor (VDR) by WT1 mediates apoptosis in mouse embryonic kidney cortex. Strong interaction between VDR and WT1 is possible because the VDR contains a WT1 consensus binding site (22, 52). The metanephric blastema and podocytes express WT1 (37). In view of the action of the vitamin D receptor in the embryonic kidney, where 1,25(OH)2D3 promotes apoptosis, it is of particular note that in the adult rat kidneys investigated in this study, if anything, 1,25(OH)2D3 prevented apoptosis (although we did not study apoptosis directly). The relevance of the 1,25(OH)2D3-reduced apoptosis in the embryonic kidney is particularly puzzling because in VDR knockout mice mouse embryonic kidney cortex. Strong interaction between WT1 and VDR is possible because the VDR contains a WT1 glycans (38, 39), respectively. Evidence for detachment is provided by the observation that vital podocytes can be cultured from the urine of animals (19) or patients (13) with progressive renal disease. With respect to apoptosis, a particular comment is appropriate: it is known that activation of the vitamin D receptor (VDR) by WT1 mediates apoptosis in mouse embryonic kidney cortex. Strong interaction between VDR and WT1 is possible because the VDR contains a WT1 consensus binding site (22, 52). The metanephric blastema and podocytes express WT1 (37). In view of the action of the vitamin D receptor in the embryonic kidney, where 1,25(OH)2D3 promotes apoptosis, it is of particular note that in the adult rat kidneys investigated in this study, if anything, 1,25(OH)2D3 prevented apoptosis (although we did not study apoptosis directly). The relevance of the 1,25(OH)2D3-reduced apoptosis in the embryonic kidney is particularly puzzling because in VDR knockout mice or vitamin D binding protein knockout mouse, no abnormality of renal structure was noted (17, 43). This discrepancy is unexplained, but there have been speculations on a role of nonreceptor-mediated mechanisms and poten-tial alternative vitamin D metabolites.

Injury to podocytes in the solvent-treated animals is sug-gested by the findings of abnormal ultrastructure with signs of cell activation and degeneration (i.e., increased cytoplasmic vacuolization, pseudocyst formation, loss or flattening of foot processes, bridging of podocyte cytoplasm, etc.), increased podocyte volume, changed cytoskeleton expression pattern with appearance of desmin, and expression of PCNA. The latter observation may appear paradoxical in view of the fact that podocytes are postmitotic cells (30). However, it has recently been shown that PCNA expression may reflect cell activation rather than proliferation with full completion of the cell cycle (15). This interpretation is rendered more likely by the present observation of upregulation of p27 in glomerular cells. The increased glomerular expression of the cell cycle-inhibitory compound p27 after 1,25(OH)2D3 treatment is in line with observations in lymphocytes (34). It further confirms several studies which showed that 1,25(OH)2D3 inhibits cell proliferation in malignant (29) as well as nonmalignant cells (3). This effect of 1,25(OH)2D3 (23) and 1,25(OH)2D3 analogs (34, 46) is dependent on the presence of the VDR. It is mediated by an increased expression of the CDK-inhibitory proteins p21 and p27, respectively, inducing a G1 block in the cell cycle sec-ondary to reduction of CDK2 and CDK6 activity. This obser-vation is in line with recent findings of Petermann and co-workers (36) that podocyte injury causes upregulation of cell cycle proteins, e.g., phosphorylated histone 3, cdc2, and cyclin B1 and B2, but at the same time also of CDK-inhibitory proteins particularly p21 and p27. As a result, podocytes do not complete the cell cycle and do not undergo cytokinesis. This has been attributed to the accumulation of DNA damage, inhibiting podocyte proliferation at G1/S and G2/M phases by increased p21.

Several points in the design and methodology of the present study deserve comment. The dose of 1,25(OH)2D3 increased serum Ca in SNX than in sham-operated controls but was not affected by treatment with 1,25(OH)2D3.

In conclusion, the present study documents important direct or indirect effects of 1,25(OH)2D3 on podocytes in a standard model of nonimmune progressive renal damage as illustrated by amelioration of morphological and immunohistochemical indicators of podocyte injury. These findings may explain blood pressure-independent attenuation of progression by ac-tive vitamin D.

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GRANTS

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REFERENCES


