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

Alexander Kuhlmann,¹ Christian S. Haas,² Marie-Luise Gross,³ Udo Reulbach,⁵ Marc Holzinger,¹ Ute Schwarz,³ Eberhard Ritz,² and Kerstin Amann¹

¹Departments 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

Submitted 3 September 2003; accepted in final form 26 October 2003

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 antiproliferative 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 100 g body wt⁻¹·day⁻¹·sc). Blood pressure (BP) and albuminuria were measured. After 16 wk, the remnant renal tissue was perfusion fixed and morphometric and stereological measurements were carried out. The expression of proliferating cellular antigen (PCNA), cyclin-dependent kinase inhibitor p27, Wilms tumor gene (WT1), and desmin, a marker of early podocyte damage, was assessed by immunohistochemistry. 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 x 10⁶ μm³) than in sham rats (1.44 ± 0.17 and 1.28 ± 0.14 x 10⁶ μm³); it was significantly (P < 0.05) lower in SNX+1,25(OH)₂D₃ rats (1.81 ± 0.16 x 10⁶ μm³). The main finding was a significantly higher number of podocytes in SNX+1,25(OH)₂D₃ (88 ± 9) and sham (98 ± 17) compared with SNX+solvent rats (81 ± 8). 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) inhibitory 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 (UF-1) (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.

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.
Araldite. Five of the resin blocks were randomly chosen, from which 3 mm² blocks were selected by area weight, sampling for embedding in Epon. After dextran infusion, the vascular system was perfused with 0.2% buffered formalin and embedded in paraffin. For immunohistochemistry, 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 (Aglom) and volume density (Vglom) of the glomerular tuft and medulla as well as the number of glomeruli per area (Nglom) were measured using a Zeiss eyepiece (Integration plate II; Zeiss) and the point-counting method (Pcount = Aglom × A) at a magnification of ×400 (1, 54, 55). The total area of the glomerular tuft (Aglom) was determined as Aglom = Arenal × A cortex (1). The number of glomeruli per volume (Nglom) and the volume density (Vglom) of glomeruli was determined using the formula Nglom = k/β × Nrenal/Vrenal 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: Nrenal = Nglomeruli × Vrenal/Vcortex. The mean glomerular tuft volume (Vglomeruli) was determined according to Vglomeruli = β/k × Aglom, 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 transects per area of the glomerular tuft). The total area of glomerular capillaries was derived from Arenal × Aglom. The filtration area (FF) was calculated according to the total area of glomerular capillaries times total number of glomeruli per kidney. The total number of glomeruli per kidney was determined in paraffin sections as described above.

**Immunohistological Investigations**

Immunohistochernistry 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 WT1 (rabbit polyclonal; dilution 1:300; Santa

---

**Table 1. Animal data**

<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>SCreat, mmol/l</th>
<th>Sura, mg/dl</th>
<th>SCreat, mg/dl</th>
</tr>
</thead>
<tbody>
<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>
</tr>
<tr>
<td>Sham + 1,25(OH)₂D₃ (n = 7)</td>
<td>502 ± 31.3</td>
<td>0.90 ± 0.27</td>
<td>116 ± 9.8</td>
<td>2.35 ± 0.07</td>
<td>85.6 ± 23.2</td>
<td>1.02 ± 0.26</td>
</tr>
<tr>
<td>SNX + solvent (n = 6)</td>
<td>478 ± 39.2</td>
<td>2.20 ± 0.51</td>
<td>126 ± 8.0†</td>
<td>1.93 ± 0.08†</td>
<td>181 ± 50.7†</td>
<td>2.13 ± 0.90†</td>
</tr>
<tr>
<td>SNX + 1,25(OH)₂D₃ (n = 8)</td>
<td>459 ± 11.6</td>
<td>1.94 ± 0.20</td>
<td>128 ± 13.9*</td>
<td>2.31 ± 1.09†</td>
<td>156 ± 22.2†</td>
<td>1.80 ± 0.29†</td>
</tr>
<tr>
<td>ANOVA</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SD. n, No. of rats; SCreat, Sura, and SCreat: 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.

---
Fig. 1. Effect of 1,25(OH)₂D₃ treatment on length density (Lₒ) of glomerular capillaries. Lₒ 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.

Cruz Biotechnology). The streptavidin-biotin method with fast red or dianaminobenzidin (DAB) as chromogen was used as described in detail (10, 45). Negative controls were performed by omitting the primary antibody. To reduce staining variability, all sections are stained in one procedure. In 50 glomeruli/kidney, the number of PCNA+, p27+ and WT1+ cells of the entire glomerulus as well as the number of PCNA+ cells per glomerular and per tubulointerstitial area were counted (magnification ×400). In addition, immunohistochemical staining for desmin was performed (mouse monoclonal antibody, dilution 1:400; DAKO) and investigated as a marker of podocyte degeneration or activation, respectively. For analysis of desmin immunohistochemistry, the capillary tuft was divided into 4 quarters and the following scoring system was used: 0, no expression; 1, desmin-positive cells in 1 quarter; 2, desmin-positive cells in 2 quarters; 3, desmin-positive cells in 3 quarters; and 4, desmin-positive cells all over the capillary tuft. The damage score was then calculated as [(score 0 + score 1 + score 2 + score 3 + score 4) / 4] + 15 (10).

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. 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.

AJP-Renal Physiol • VOL 286 • MARCH 2004 • www.ajprenal.org
The number of PCNA-positive glomerular cells was significantly ($P < 0.05$) higher in solvent-treated SNX ($2.10 \pm 0.76 \times 10^4$) compared with sham+solvent ($2.00 \pm 0.88 \times 10^4$) and SNX+solvent rats ($1.95 \pm 0.77 \times 10^4$) (Table 3). This was due to the fact that glomerular enlargement in SNX+solvent 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+solvent 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 significant ($P < 0.001$) lower number of WT1-positive cells per glomerulus in SNX+solvent ($492 \pm 0.11$) compared with SNX+1,25(OH)$_2$D$_3$ rats ($7.0 \pm 0.13$). In parallel, mean podocyte volume was significantly higher in SNX+solvent 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$_3$.

**Table 3. Effect of 1,25(OH)$_2$D$_3$ on glomerular cells**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Podocyte No./Glomerulus</th>
<th>Mean Podocyte Volume, $\mu$m$^3$</th>
<th>Mean Mesangial Cell No./Glomerulus</th>
<th>Mean Endothelial Cell No./Glomerulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham+solvent</td>
<td>$93 \pm 9.3^*$</td>
<td>$4.207 \pm 732$</td>
<td>$53.7 \pm 7.1$</td>
<td>$201 \pm 24.1$</td>
</tr>
<tr>
<td>Sham+1,25(OH)$_2$D$_3$</td>
<td>$98 \pm 11.7$</td>
<td>$4.023 \pm 1.095$</td>
<td>$52.1 \pm 9.8$</td>
<td>$199 \pm 42.7$</td>
</tr>
<tr>
<td>SNX+solvent</td>
<td>$81 \pm 8.7^*$‡§</td>
<td>$5.771 \pm 2.922^*$‡§</td>
<td>$103 \pm 7.6^*$‡§</td>
<td>$383 \pm 34^*$‡§</td>
</tr>
<tr>
<td>SNX+1,25(OH)$_2$D$_3$</td>
<td>$88 \pm 9.4^*$§</td>
<td>$3683 \pm 905^*$§</td>
<td>$67.7 \pm 10.3^*$§§</td>
<td>$247 \pm 54.9^*$§§</td>
</tr>
<tr>
<td>ANOVA</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
</tr>
</tbody>
</table>

Values are means ± SD. n, No. of rats; $^*$Values in $n = 7$ animals due to an outlier (42). $^\dagger P < 0.05$ vs. sham+solvent rats. $^\ddagger P < 0.05$ vs. sham+1,25(OH)$_2$D$_3$ rats. $^\S P < 0.05$ vs. SNX+solvent rats.
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 syncitia, 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, A 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 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. The 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)_2D_3.

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)_2D_3 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 dystro-
glycan (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
podocyte precursors in immature glomeruli as well as mature
podocytes express WT1 (37). In view of the action of 1,25(OH)2D3 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
mice, no abnormality of renal structure was noted (17, 43).
This discrepancy is unexplained, but there have been specula-
tions on a role of nonreceptor-mediated mechanisms and po-
tential 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-inhibi-
tory 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 prolifera-
tion 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 Pettermann 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 rats to some extent, but not into the
hypercalcemic range. We used a dose of 1,25(OH)2D3 that
restored plasma 1,25(OH)2D3 levels to the physiological range.
Consequently, the results reflect physiological actions rather
than pharmacological consequences of 1,25(OH)2D3. Inten-
tionally, only a modest amount of cortical tissue was resected
so that the number of nephrons was only reduced by ~60% as
documented for this resection protocol (1). This was done to
avoid confounders such as severe hypertension, severe hyper-
parathyroidism, metabolic acidosis, anemia, etc. In particular,
blood pressure as a potential confounder for glomerular injury
was higher 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.

ACKNOWLEDGMENTS
The skilful technical assistance of Z. Antoni, M. Klewer, P. Rieger, S.
Söllner, M. Weckbach, and H. Ziebart is gratefully acknowledged.

GRANTS
This work was supported by the Deutsche Forschungsgemeinschaft (SFB
423, project B8).

REFERENCES
E. Effect of ramipril, nifedipine, and moxonidine on glomerular morphol-
ogy and podocyte structure in experimental renal failure. Nephrol Dial
2. Branisteau DD, Leenaerts P, van Damme B, and Bouillon R. Partial
prevention of active Heymann nephritis by 1α,25 dihydroxyvitamin D3.
3. Casado M, Martin M, Munoz A, and Bernal J. Vitamin D3 inhibits
proliferation and increases c-myc expression in fibroblasts from psoriatic
4. Chen HC, Chen CA, Guh JY, Chang JM, Shin SJ, and Lai YH.
Altering expression of αβ3 integrin on podocytes of human and rats with
5. Coimbra TM, Janssen U, Grüne HJ, Ostendorf T, Kunter U, Schmidt
H, Brabant G, and Floege J. Early events leading to renal injury in obese
Adhesion of rat glomerular epithelial cells to extracellular matrices: role of
7. DeLuca HF. The vitamin D story: a collaborative effort of basic science
8. Fleoge J, Alpers CE, Sage EH, Brabant G, and Floege J. Early events
leading to renal injury in obese Zucker (fatty) rats with type II diabetes.
9. Fries JW, Sandstrom DJ, Meyer TW, and Rennke HG. Glomerular
hypertrophy and epithelial cell injury modulate progressive glomerulo-
naviciene A, Kuhlmann A, Münther K, Ritz E, and Amann K. ACE


