Effect of ramipril, nifedipine, and moxonidine on glomerular morphology and podocyte structure in experimental renal failure


Departments of Pathology Heidelberg and Darmstadt, Department of Internal Medicine Ruperto Carola University Heidelberg, Germany

Abstract

Background. Experimental renal failure causes structural alterations of the kidney. It is still unresolved how these changes are modified by antihypertensive treatment.

Purpose of the study. To examine the effects of different antihypertensive agents (ramipril, nifedipine, moxonidine) mainly on glomerular geometry, cell number, cell morphology, and capillarization, in a subtotal nephrectomy model of renal failure.

Material and methods. Sham-operated male SD rats and subtotally nephrectomized (SNX) ad libitum-fed rats were examined. Groups of 8-10 SNX rats were left untreated or were treated with ramipril (0.5 mg/kg b.w. per day), nifedipine (30 mg/kg b.w. per day) or moxonidine (10 mg/kg b.w. per day) respectively. After perfusion fixation the kidneys were examined using stereological techniques.

Results. Systolic blood pressure (by tail plethysmography) was $110\pm13$ mmHg in sham-op and $119\pm9$ in SNX. It was effectively and comparably reduced below normal values by ramipril ($89\pm11$ mmHg), nifedipine ($98\pm23$ mmHg) and moxonidine ($92\pm11$ mmHg). The glomerulosclerosis index (GSI) was significantly increased in SNX versus sham-op; it was similarly decreased by ramipril and moxonidine but less so by nifedipine. Vascular damage (preglomerular vessels) was reduced by all treatments whereas tubulointerstitial damage was significantly reduced only by ramipril and moxonidine. Mean glomerular tuft volume was increased in SNX compared to sham-op, controls and was normalized only by ramipril treatment. Glomerular cells were differentially affected by the three antihypertensive agents. After subtotal nephrectomy an increase in podocyte volume and mesangial cell number per glomerulus was noted. Nifedipine, and to a lesser extent ramipril, prevented mesangial cell hyperplasia. In contrast, only the ACE inhibitor ramipril, but not nifedipine or moxonidine prevented podocyte abnormalities, particularly podocyte hypertrophy.

Conclusions. (i) Despite comparable reduction in systolic blood pressure, different classes of antihypertensive agents had diverse effects on renal damage in subtotally nephrectomized rat. This observation is consistent with specific, non-hemodynamic actions of antihypertensives. (ii) Glomerular and tubulointerstitial damage are prevented by treatment with ACE inhibitors and antisympathotonic agents, but not with the calcium antagonist nifedipine. In contrast, renal vascular changes were also prevented by nifedipine. (iii) Only ACE inhibitors effectively inhibited podocyte hypertrophy and mesangial cell hyperplasia. Whether the superior effect of ACE inhibitors on glomerulosclerosis is related to inhibition of glomerular growth and podocyte hypertrophy as well as preservation of podocyte structure, or whether these findings are merely a passive reflection of greater efficacy, remains unresolved.

Key words: ACE inhibitors; calcium-channel blockers; sympatholytic agents; kidney; renal failure; podocyte growth

Introduction

Experimental renal failure by either arterial ligation or surgical ablation leads to marked structural alterations of the remaining renal tissue, i.e. glomerular hypertrophy, glomerulosclerosis, vascular lesions and tubulointerstitial damage [1,2]. Apart from hypertension, several non-haemodynamic factors are thought to play a role in the development of such renal changes, e.g. glomerular growth, activation of the local renin-angiotensin systems, deposition of advanced glycosylation end products (AGE), inappropriate activation of the sympathetic nerve system, elevated levels of parathyroid hormone, to name only a few.

It is well known that systemic hypertension accelerates progression of renal failure [3]. Conversely, antihypertensive treatment prevents progression [4]. Recent studies [5-7] demonstrated class-specific effects of different antihypertensive agents, particularly ACE
inhibitors. Such actions can possibly be dissociated from lowering of (casual) blood pressure [4,5]. This observation supports the notion that non-haemodynamic actions are involved.

The questions of whether nephroprotective actions of different antihypertensives are associated with, or related to, effects on glomerular cellularity and/or capillarization have not yet been addressed.

We investigated whether antihypertensive treatment with equipotent doses of either an ACE inhibitor (ramipril), a dihydropyridine type calcium antagonist (nifedipine) or a central sympatholytic agent (moxonidine) prevented or ameliorated to a similar extent the development of structural abnormalities in the kidneys of subtotally nephrectomized rats. We used doses which were equipotent at least with respect to (casual) systemic blood pressure.

Apart from well-known indices of renal damage (glomerulosclerosis index, indices of tubulointerstitial and vascular damage) stereological techniques were used to further investigate changes in (i) glomerular capillarization, (ii) glomerular cell density and (iii) ultrastructure of podocytes, mesangial cells or endothelial cells.

**Subjects and methods**

**Animals**

Male 300 g Sprague–Dawley rats (SD) were housed in single cages at constant room temperature (20 °C) and humidity (75%) under a controlled light–dark cycle. The rats were fed a diet containing 40 g protein and 0.6 g NaCl per 100 g (Altromin Co., Lage/Lippe, Germany). After a 3-day adaptation period the animals were randomly allotted to five groups:

1. Control group: sham-operation (Sham)
2. Subtotally nephrectomized group: no treatment (SNX)
3. Subtotally nephrectomized group: nifedipine treatment (N) (0.5 mg/kg/day)
4. Subtotally nephrectomized group: ramipril treatment (R) (0.5 mg/kg/day)
5. Subtotally nephrectomized group: moxonidine treatment (M) (10 mg/kg/day)

Group 1 was sham operated and left untreated whereas groups 2–5 underwent two-step subtotal nephrectomy with weight controlled removal of 75% of renal (cortical) tissue as described in detail elsewhere [7]. Forty-eight hours after the second operation antihypertensive treatment was started in groups 2–5. Treatment comprised ramipril 2.8 mg/l in the drinking fluid (new solutions were prepared every 48 h), nifedipine 400 mg/kg in food pellets (taking care to prevent exposure to light) and moxonidine 130 mg/kg in food pellets. Apart from well-known indices of renal damage (glomerulosclerosis index, indices of tubulointerstitial and vascular damage) stereological techniques were used to further investigate changes in (i) glomerular capillarization, (ii) glomerular cell density and (iii) ultrastructure of podocytes, mesangial cells or endothelial cells.

Indices of renal damage (glomerular sclerosis, tubulointerstitial and vascular damage)

Percentage of sclerosed area of glomerular tuft as parameter of glomerular damage was determined in at least 100 glomeruli per animal according to Raij et al. [10] on PAS stained paraffin sections at a magnification of 1000 using planimetry and a semiautomatic image analysing system (IBAS II, Kontron Co., Eching, Germany). Tubulointerstitial (tubular atrophy, dilatation, casts, interstitial inflammation, and fibrosis) and vascular changes as parameters of interstitial and vascular damage were determined using a semi-quantitative scoring system according to Veniant et al. [11] on HE stained paraffin sections at a magnification of 100. Ten fields per kidney were randomly sampled and graded as follows: grade 0, no changes; grade 1, lesions involving less than 25%; grade 2, lesions affecting 25–50%; and grade 3, lesions involving more than 50% of the field. The resulting index in each animal was expressed as a mean of all scores obtained.

Glomerular geometry

Paraffin sections (light-microscopy using various magnifications). Area (A<sub>R</sub>) and volume density (V<sub>V</sub>) of the renal cortex and medulla as well as the number of glomeruli per area (N<sub>g</sub>) were measured using a Zeiss eyepiece (Integrationplate II, Zeiss Co., Oberkochen, Germany) and the point counting method (P<sub>p</sub> = A<sub>x</sub> = V<sub>V</sub>) at a magnification of ×400 [8,9]. The number of glomeruli per area (N<sub>g</sub>) was then corrected for tissue shrinkage (45%).

Total cortex volume (V<sub>cortex</sub>) was derived from kidney mass divided by specific weight of the kidney (1.04 g/cm<sup>3</sup>) times the volume density of the cortex.

Glomerular geometry was analysed as follows:

Volume density (V<sub>V</sub>) of glomeruli and area density of glomerular tuft (A<sub>x</sub>) were measured by point counting according to P<sub>p</sub> = A<sub>x</sub> = V<sub>V</sub> [8,9] at a magnification of ×400 on HE sections.

Total area of glomerular tuft (A<sub>T</sub>) was then determined as A<sub>T</sub> = A<sub>x</sub> × A<sub>cortex</sub>.
The number of glomeruli per volume (Nv) was then derived from glomerular area density (N_A) and the volume density (V_v) of glomeruli using the formula:
\[ N_v = \frac{k}{\beta} \frac{N_A}{V_v} \]
with \( k = 1 \) and \( \beta = 1.382 \).

The total number of glomeruli was derived from the total volume of the renal cortex and the number of glomeruli per cortex volume: \( N_{\text{glomeruli}} = N_v \times V_{\text{cortex}} \).

The mean glomerular tuft volume was determined according to
\[ v = \frac{\beta}{k} \frac{1}{A_T^{1.5}} \]
with \( \beta = 1.382 \) and \( k = 1.1 \) [8,9].

**Glomerular capillaries and cells**

Semithin sections (light-microscopy, magnification \( \times 1000 \), oil immersion). Length density (L_v) of glomerular capillaries was determined according to the standard stereological formula \( L_v = 2 Q A \) (with \( Q A \) being the number of capillary transsects per tissue area).

Glomerular cellularity (podocytes, mesangial and endothelial cells) was assessed by stereological techniques described by Weibel [8] using the point counting method and a Zeiss eyepiece (Integrationsplatte II, Zeiss Co., Oberkochen, Germany) containing a 100-point grid on semithin sections in at least 30 glomeruli per animal, using the formula
\[ N_v = \frac{k}{\beta} \frac{N_A}{V_v} \]

\( \beta \) for podocytes was assumed to be 1.5 and \( k = 1 \) [12,13]. For mesangial and endothelial cells \( \beta = 1.4 \) and \( k = 1 \) [14].

Mean capillary cross sectional area was determined using the following equation [15]:
\[ k = \frac{A_A}{Q_A} \]

Total area of glomerular capillaries was derived from \( A_A \times A_T \).

Filtration area was calculated according to total area of glomerular capillaries times total number of glomeruli per kidney.

**Statistics**

Data are given as mean ± standard deviation. Kruskal–Wallis test and one-way ANOVA respectively were chosen for analysis of variance, followed by Duncan’s multiple-range test to determine whether the differences between the groups were significant or not. The results were considered significant when probability of error (P) was less than 0.05.

**Results**

**Animal data**

After 8 weeks of uraemia (see Table 1) plasma urea concentration was significantly higher in subtotally nephrectomized animals and was even more elevated in ramipril-treated animals versus untreated SNX animals. The animals were not anaemic. Body weight was slightly but not significantly lower in SNX animals fed ad libitum. It was significantly lower in the ramipril- and the moxonidine-treated groups. Casual systolic blood pressure (by tail plethysmography) was slightly but not significantly higher in SNX animals and had (intentionally) been lowered below the values of sham-operated controls in the three intervention groups. Left kidney weight was higher in SNX and was significantly lowered by antihypertensive treatment with ramipril and moxonidine whereas nifedipine did not prevent the increase of kidney weight after subtotal nephrectomy. Left kidney/body weight was significantly and comparably increased in all subtotally nephrectomized groups. The left ventricular weight/body weight ratio was increased after subtotal nephrectomy. This increase was prevented by ramipril treatment.

**Structural changes of the kidney**

**Glomerular, tubulointerstitial, and vascular changes**

By surgical ablation the total number of glomeruli per two kidneys (90 000) was reduced to around 21 000

**Table 1. Animal data (values as mean ± standard deviation)**

<table>
<thead>
<tr>
<th></th>
<th>Blood pressure (mmHg)</th>
<th>Plasma urea (mg/dl)</th>
<th>Body weight (g)</th>
<th>Left ventricular weight/body weight (mg/g)</th>
<th>Weight of left kidney (g)</th>
<th>Left kidney weight/body weight (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-op (n=10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subtotal nephrectomy (SNX) (n=9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNX + ramipril (n=8)</td>
<td>110±13</td>
<td>54.7±4.7</td>
<td>558±28.8</td>
<td>1.90±0.15</td>
<td>2.15±0.16</td>
<td>3.85±0.26</td>
</tr>
<tr>
<td>SNX + moxonidine (n=8)</td>
<td>119±9</td>
<td>118±11.3</td>
<td>534±42.2</td>
<td>2.31±0.19</td>
<td>2.85±0.40</td>
<td>5.35±0.47</td>
</tr>
<tr>
<td>SNX + nifedipine (n=9)</td>
<td>98±12</td>
<td>142±12</td>
<td>507±44.6</td>
<td>2.09±0.38</td>
<td>2.98±0.44</td>
<td>5.90±0.89</td>
</tr>
<tr>
<td>Analysis of variance</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

1In perfusion fixed tissue.

BLOOD PRESSURE: SYSTOLIC PRESSURE BY TAIL PLETHYSMOGRAPHY (MEAN OF 3 MEASUREMENTS AT DAY 31, 41, 54).

\* P<0.05 vs. untreated SNX; \# P<0.05 vs. sham-operated controls.
in the remnant kidney. The reduction of renal mass was by chance greater in the ramipril-treated subtotally nephrectomized rats than in the other SNX groups (Table 2a).

The glomerulosclerosis index (as percentage of tuft area) was significantly higher in SNX than in sham-op and was comparably decreased by antihypertensive treatment with ramipril and moxonidine whereas nifedipine was less effective. The index of vascular damage was increased in untreated SNX and was similarly lowered by all treatments. In contrast, the index of tubulointerstitial damage was increased after subtotal nephrectomy but was significantly lowered only by ramipril and moxonidine. The mean glomerular tuft volume was significantly increased in untreated subtotally nephrectomized rats (Figure 1a,b). Such increase was only prevented by ramipril treatment.

**Glomerular capillarization and cells**

Mean capillary cross-sectional area was increased in untreated SNX and in all groups subjected to antihypertensive treatment. In contrast, total filtration area per left kidney and capillary length density (L_v), i.e. capillary length per unit volume of tuft tissue, were significantly decreased in SNX and were not normalized by treatment (Table 2b).

The mean podocyte volume was significantly increased in SNX whereas podocyte number was significantly decreased. Such podocyte hypertrophy was almost completely prevented by ramipril treatment, but was unaffected by the other treatments (Table 2c).

Mesangial cell volume was unchanged after subtotal nephrectomy, but mesangial cell number per glomerulus was increased. Such mesangial cell hyperplasia was completely prevented by nifedipine and in part by ramipril treatment.

Mean endothelial cell volume was not changed after subtotal nephrectomy, but the number of endothelial cells per glomerulus was significantly increased. This went in parallel with a similar fractional increase in tuft volume. The increase in endothelial cell number was only prevented by moxonidine treatment.

**Ultrastructural changes**

In untreated subtotally nephrectomized rats (Figure 2b) glomeruli showed marked expansion of mesangial matrix, mesangial cell hyperplasia and podocyte hypertrophy with rarefication and fusion of the foot processes, podocyte ballooning, and separation from the basement membrane compared with sham-operated controls (Figure 2a). Podocytes lost their adhesive phenotype and assumed the appearance of rounded off cells (Figure 2b).

**Table 2a. Glomerular morphology and indices of renal damage**

<table>
<thead>
<tr>
<th>Group</th>
<th>Total number of glomeruli per left kidney</th>
<th>Mean volume of glomerular tuft (10⁶ μm³)</th>
<th>Proportion of sclerosed area of glomerular tuft (%)</th>
<th>Index of tubulointerstitial damage (10⁻⁶ mm²)</th>
<th>Index of vascular damage (10⁻⁶ mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>44956 ± 3396*</td>
<td>1.47 ± 0.11*</td>
<td>13.6 ± 2.7*</td>
<td>0.73 ± 0.15*</td>
<td>1.1 ± 0.16*</td>
</tr>
<tr>
<td>SNX</td>
<td>21671 ± 3727*</td>
<td>2.63 ± 0.41#</td>
<td>34.9 ± 8.3%</td>
<td>1.73 ± 0.13#</td>
<td>1.71 ± 0.35#</td>
</tr>
<tr>
<td>SNX + ramipril (n = 8)</td>
<td>15157 ± 4860**</td>
<td>1.82 ± 0.39*</td>
<td>13.4 ± 2.9%</td>
<td>1.36 ± 0.10*</td>
<td>1.36 ± 0.20*</td>
</tr>
<tr>
<td>SNX + moxonidine (n = 8)</td>
<td>19480 ± 3336§</td>
<td>2.34 ± 0.33§</td>
<td>12.5 ± 2.1%</td>
<td>1.31 ± 0.16§</td>
<td>1.36 ± 0.10*</td>
</tr>
<tr>
<td>SNX + nifedipine (n = 9)</td>
<td>22930 ± 4818§</td>
<td>2.65 ± 0.67§</td>
<td>20.1 ± 2.9%</td>
<td>1.73 ± 0.23§</td>
<td>1.35 ± 0.29§</td>
</tr>
<tr>
<td>Analysis of variance</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

#P < 0.05 versus control; *P < 0.05 versus untreated SNX.

**Table 2b. Glomerular capillaries**

<table>
<thead>
<tr>
<th>Group</th>
<th>Capillary mean cross-sectional area (μm²)</th>
<th>Length density of glomerular capillaries (L_v) (mm/mm³)</th>
<th>Total capillary length per glomerulus (mm)</th>
<th>Total filtration area (cm²) per left kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>144 ± 13.7*</td>
<td>5569 ± 463*</td>
<td>6.67 ± 0.817*</td>
<td>236.0 ± 33.37*</td>
</tr>
<tr>
<td>SNX</td>
<td>191 ± 30.6*</td>
<td>4182 ± 563*</td>
<td>10.271 ± 2.232*</td>
<td>161.5 ± 34.50*</td>
</tr>
<tr>
<td>SNX + ramipril (n = 8)</td>
<td>230 ± 29.3**</td>
<td>4047 ± 599*</td>
<td>9.419 ± 1.349*</td>
<td>160.0 ± 23.38*</td>
</tr>
<tr>
<td>SNX + moxonidine (n = 8)</td>
<td>220 ± 26.9§</td>
<td>4059 ± 513*</td>
<td>9.368 ± 1.177*</td>
<td>150.5 ± 26.38*</td>
</tr>
<tr>
<td>SNX-nifedipine (n = 9)</td>
<td>215 ± 31.0*</td>
<td>3726 ± 529*</td>
<td>8.227 ± 1.506*</td>
<td>174.43 ± 51.67*</td>
</tr>
<tr>
<td>Analysis of variance</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

#P < 0.05 versus control; *P < 0.05 versus untreated SNX.
Discussion

This study examined the effect of antihypertensive agents on the development of glomerulosclerosis after subtotal nephrectomy. The salient features are the following:

Glomerulosclerosis, and in parallel tubulointerstitial damage, was similarly reduced by the ACE-inhibitor ramipril and the antisympathicotonic agent moxonidine, but less effectively prevented by the calcium-channel blocker nifedipine. In contrast, all agents were similarly effective in preventing vascular lesions.

Subtotal nephrectomy led to an increase in left kidney weight, which was prevented by ramipril and moxonidine treatment. In contrast, left kidney weight significantly and comparably increased in all subtotal nephrectomized groups. Subtotal nephrectomy led to an increase in left kidney weight, which was prevented by ramipril and moxonidine treatment. In contrast, left kidney weight significantly and comparably increased in all subtotal nephrectomized groups.

Left kidney weight, which was prevented by ramipril and moxonidine treatment. In contrast, left kidney weight significantly and comparably increased in all subtotal nephrectomized groups.
length was noted. In contrast, the number of podocytes decreased, while podocyte volume was dramatically increased in untreated subtotally nephrectomized rats. Ramipril had a beneficial effect on podocyte size and ultrastructure.

In principle the above findings confirm reports documenting that different antihypertensive agents have diverse effects on glomerular lesions in glomerular injury models. For example, Brunner et al. [16] observed that enalapril but not verapamil conferred specific benefit with respect to development of glomerulosclerosis. Good arguments have been offered that the effects of ACE-inhibition are, at least in part, independent of glomerular hyperfiltration and hypertension [3]. Specifically, inhibition of glomerular growth may be involved [4]. An effect of calcium-channel blockers on glomerular growth was also noted in some studies but this was not consistently confirmed [15,16]. The above results are in general agreement with such observations. We emphasize that in our study the effect of the antisympathetic agent moxonidine on glomerulosclerosis was as pronounced as that of the ACE inhibitor ramipril. This may be of interest because sympathetic overactivity, presumably triggered by afferent signals originating in the kidney, has been documented in models of renal damage [18,19].

We are aware of several limitations of the study. First, for logistic reasons only one dose of antihypertensive agents could be used and the dose–response relationship was not investigated. Treatment started at the time of subtotal nephrectomy so that we addressed the issue of prevention and not the issue of reversal of lesions.

Second, sham-operated animals were not given antihypertensive treatment. This deficit is mitigated, however, by the fact that antihypertensive treatment with the comparable agent enalapril failed to affect renal structure of control animals in a previous study from this laboratory [7]. Nevertheless the protocol does not permit definite exclusion of a potential specific effect of hypotension.

Third, the total number of glomeruli in ramipril-treated rats by chance was significantly lower than in the other SNX groups. Although ramipril-treated
animals had less morphological injury, we cannot exclude that the different degree of renal ablation (by numbers of glomeruli) may interfere with the interpretation of the study.

Fourth, we are aware that blood-pressure measurements by tail plethysmography have definite limitation [20]. To avoid the argument that differences between antihypertensive treatments were related to different degrees of control of hypertension, we elected to reduce blood pressure in all intervention groups to levels below that in sham-operated control animals. This manoeuvre provided safety against the known effects of intermittent elevation of blood pressure in subtotal nephrectomized animals [20]. The use of multiple parallel groups precluded a pair-feeding protocol. Animals were given free access to food and consequently differed with respect to final body weight. Lower final body weight in ramipril- and moxonidine-treated animals went in parallel with higher rise in plasma urea concentration in the treated subtotally nephrectomized animals. Presumably because of disturbed renal autoregulation after subtotal nephrectomy, plasma urea concentrations tended to be higher in the subtotally nephrectomized groups with overcorrection of hypertension. We cannot exclude the alternative hypothesis that these animals were catabolic because of lower food intake. We would like to emphasize that these non-hypertensive subtotally nephrectomized animals were not in advanced terminal uraemia.

Several observations deserve specific comment. As anticipated compensatory growth of the residual kidney occurred after subtotal nephrectomy. In contrast to the observation of Dworkin et al. [21,22], however, compensatory renal growth was not affected by nifedipine. We cannot exclude that this is related to the use of different animal strains and different renal damage models. Overall compensatory kidney growth as indicated by left kidney weight/body weight ratio was not significantly affected by ramipril and moxonidine.

**Glomerulosclerosis.** ACE inhibition and antisympathetic treatment dramatically prevented development of glomerulosclerosis after subtotal nephrectomy. This agrees with previous observations of this laboratory [7] and other authors [3–6]. With respect to preventing glomerulosclerosis nifedipine was much less effective [16,23]. The changes in tubulointerstitial damage index paralleled those in glomerulosclerosis in a remarkably close fashion.

**Glomerular enlargement.** Substantial glomerular hypertrophy was noted after subtotal nephrectomy and this was effectively prevented by ramipril only. This observation reinforces the conclusion of a previous study that ACE inhibitors specifically interfere with compensatory glomerular growth [7].

**Glomerular capillaries.** More detailed structural analysis of glomeruli showed that capillary length per glomerular tuft volume was significantly reduced after subtotal nephrectomy, presumably reflecting capillary occlusion due to advanced glomerulosclerosis. One further consideration is that capillary rarefaction is accompanied by capillary dilatation [24,25]. In parallel, total filtration area was decreased after subtotal nephrectomy. Total capillary length per glomerulus increased in parallel with glomerular volume. We cannot exclude that capillary subdivision occurs [26]. Length density of glomerular capillaries and total capillary length were not changed by any antihypertensive treatment. In particular we could not demonstrate a specific effect of the ACE-inhibitor on angiotensin on angiogenesis [27,28]. In parallel, capillary cross-sectional area was significantly increased after subtotal nephrectomy and was not altered by antihypertensive treatment. The observation of capillary dilatation is in good agreement with findings of Bidani et al. [24] and Daniels and Hostetter [25], who demonstrated capillary dilatation following subtotal nephrectomy in normotensive and hypertensive rats respectively.

**Podocytes.** Glomerular epithelial cells (podocytes) are thought to play a pivotal role in maintaining the glomerular permselectivity [28] and in the development of glomerular scarring [29–34]. After subtotal nephrectomy, the number of podocytes in contrast to mesangial and endothelial cell number did not increase in parallel with tuft volume. This observation is in line with the concept that podocytes are postmitotic cells [13,35]. If anything the number of podocytes per glomerulus decreased, possibly because of apoptosis, desquamation, or synchiae formation. A higher number of podocyte nuclei were counted in ramipril-treated animals but we cannot exclude an artefact from counting binucleated cells. The concept of Rennke and colleagues [13,35] that podocytes are literally spread thin to cover an enlarging filtration surface area is supported by the marked decrease in the ratio podocytes/filtration surface (from 138 to 82 podocytes per mm² filtration area). A marked increase in podocyte volume and ultrastructural changes such as fusion of foot processes, ballooning, detachment from GBM and loss of adhesive phenotype were noted after subtotal nephrectomy. Podocyte morphology was almost selectively ameliorated by ramipril.

**Mesangial and endothelial cells.** In contrast to podocyte volume, mesangial and endothelial cell volumes were not significantly increased after subtotal nephrectomy. The numbers of mesangial and endothelial cells increased. Presumably this represents a harmonic increase in parallel with increased glomerular tuft volume. Mesangial cell hyperplasia was reduced by ramipril treatment and was prevented by nifedipine, despite no significant effect on tuft volume. The increase in endothelial cell number after subtotal nephrectomy, possibly reflecting capillary growth by subdivision during glomerular hypertrophy [26] was prevented by moxonidine and (in part) by nifedipine.

The striking effect of ramipril on podocyte morphology is of particular interest. Podocytes express receptors for angiotensin II, at least in vitro [29, Pavenstädt, personal communication] although this issue is still controversial [36]. These cells are extremely reactive, however, as exemplified by the presence of receptors for, and actions to endothelin I, bradykinin, histamine etc. [29]. Furthermore they secrete growth factors such...
as VEGF [37], tissue factor [38], monocyte chemotactic factor [39] etc. They also secrete extracellular matrix as well as matrix-degrading proteinase [40] and cytokines, particularly after stimulation with TGF-β or interleukin-6 [41]. The latter stimulates collagen IV secretion by podocytes on its own [41]. In addition, basic fibroblast growth factor has been shown to stimulate podocytes in vitro [42] or in vivo [43]. Floege and co-workers [44] showed that activation of podocytes, which was confirmed by PCNA staining in the experimental model of passive Heymann nephritis, was preceded by increased expression of PDGF-B chain protein and mRNA. Moreover, cultured podocytes were shown to express PDGF-B chain mRNA and the expression could be upregulated by stimulation with recombinant IL-1β [44]. These findings are of interest since PDGF-B is known to be an important factor for development of glomerulosclerosis and interstitial fibrosis.

In view of the impressive ability of podocytes to marshal mechanisms that are potentially relevant in the genesis of glomerulosclerosis [29] it is particularly relevant that podocyte abnormalities are so strikingly and selectively ameliorated by ramipril. Our observation does not permit us to distinguish cause or consequence, i.e., whether preserved podocyte structure is a passive reflection of less glomerulosclerosis or whether it is causally involved in the protection against development of glomerulosclerosis. In this context it is of note that recent findings document that direct podocyte damage in single nephron leads to albuminuria [45]. It is also very interesting that in focal segmental glomerulosclerosis, podocyte lose their adhesive phenotype [46] so that shedding of podocytes from the GBM is facilitated.

It is of further interest that the study of Pagtalunan et al. [47] does not support the hypothesis that angiotensin II by intrarenal infusion causes changes in epithelial cell or filtration slit structure. This observation differs from the findings of Olivetti et al. [48], who detected a reduction in slit length in rats receiving angiotensin II in hypertensive doses. It is conceivable that the effects of angiotensin II on podocytes depend on the model of renal damage.

Despite the fact that outcome and renal morphology in the treated groups may have been influenced by a number of different variables, the above observations indicate that different classes of antihypertensives have strikingly diverse effects on the structural abnormalities of the kidney in the subtotally nephrectomized rat. At the dose investigated ACE inhibition provided protection against (i) development of glomerulosclerosis, (ii) enlargement and structural abnormalities of podocytes, and (iii) mesangial cell proliferation.

The striking effect of ACE inhibitors on podocyte size and structure leads to the question of whether angiotensin II (and less likely bradykinin) play a role in the genesis of this lesion, which is felt to be a key step in the development of glomerulosclerosis in experimental renal failure [43].

Acknowledgements. With the support of the Deutsche Forschungsgemeinschaft (Am 93/2–1). Part of the study was also supported by grants from the Faculty of Medicine, University of Heidelberg, and Giulini Co., Hannover, Germany. The skilful technical assistance of Z. Antoni, G. Gorsberg, B. Hilbert, D. Lutz, and P. Rieger is gratefully acknowledged. The authors thank H. Derks for excellent phototechnical support and Dr. Rüdiger Wanne, Dept. of Animal Pathology, University of Munich, for his helpful advice.

References

20. Bidani AK, Griffin KA, Picken M, Lansky DM. Continuous telemetric blood pressure monitoring and glomerular injury in...


35. Renneke HG. How does glomerular epithelial cell injury contribute to progressive glomerular damage? *Kidney Int* 45; [Suppl 45]: S55–S63


Received for publication: 27.12.95
Accepted in revised form: 8.2.96