The renoprotective effect of angiotensin-converting enzyme inhibitors in experimental chronic renal failure is not dependent on enhanced kinin activity

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Introduction

Angiotensin-I-converting enzyme (ACE) inhibitors attenuate progression of renal failure both in experimental models [1-3] and in patients with glomerular disease [4,5]. Generally, two major mechanisms have been implicated to explain the effects of ACE inhibitors: (i) suppression of angiotensin II formation [6]; (ii) increase of kinin concentration via inhibition of bradykinin degradation [7,8]. Both pathways can cause a decrease in glomerular capillary pressure, proteinuria, or growth of renal cells, accompanied by increased degradation of extracellular matrix (see [9] for review). The relative roles of the two mechanisms in the renal protection conferred by ACE inhibitors has not been established, however.

Using a selective B2 bradykinin receptor antagonist, we evaluated the role of the kinin system in the renoprotective effect of ACE inhibitors in the remnant kidney model. Glomerulosclerosis was chosen as the primary study endpoint.

Subjects and methods

Male 200–300 g Sprague-Dawley rats were housed in single cages at constant room temperature (20°C) and humidity (25%) under a 12-h light/dark cycle. The rats were fed a standard diet containing 19% protein and 0.2% sodium (Altromin Co., Lage/Lippe, Germany). After a 3-day adaptation period the animals were randomly allotted to six groups. Group 1 (n=9) was sham-operated while groups 2-6 underwent a two-step subtotal (5/6) nephrectomy, i.e. left-side nephrectomy and, a week later, surgical ablation of the lower and upper poles of the right kidney (2/3 of the weight of the resected contralateral kidney). Group 2 (n=8) was then left untreated. Group 3 (n=7) received the ACE inhibitor ramipril in tap water calculated to yield a daily dose of 0.3 mg/kg. Group 4 (n=9) was treated as group 3, but the B2 bradykinin receptor antagonist HOE 140 (Hoechst AG, Frankfurt, Germany) 0.5 mg/kg/day was infused by subcutaneous osmotic minipump. Group 5 (n=9) received HOE 140 alone at the same dose. The treatment in the groups 3 to 5 was
started 48 h after the second operation. In addition group 6 (n=7) was left untreated throughout the first 4 weeks, followed by daily administration of ramipril 0.3 mg/kg from 5 to 8 week.

Systolic blood pressure was measured in conscious animals by tail plethysmography prior to operation and later at 2-week intervals. Serum creatinine concentration was assessed at the end of the experiment. The experiment was terminated after 8 weeks by retrograde perfusion fixation via the abdominal aorta as described elsewhere [10]. The kidneys were weighed and sliced in a plane perpendicular to the interpolar axis. The slices were embedded in paraffin and sectioned at 4 µm to be stained with haematoxylin/eosin and PAS.

Glomerulosclerosis index (GSI) was determined in at least 100 glomeruli per animal according to El Nahas et al. [11] on PAS-stained sections at a magnification of 400. Glomerulosclerosis severity was graded from 0 to 4 using a semiquantitative score: grade 0, normal glomeruli; grade 1, presence of mesangial expansion or mild segmental hyalinosis/sclerosis involving less than 25% of the tuft; grade 2, moderate glomerulosclerosis (25–50% of the tuft); grade 3, severe glomerulosclerosis (50–75% of the tuft), and grade 4, diffuse glomerulosclerosis, with the involvement of more than 75% of the tuft. GSI was calculated for each animal as the mean value of the scores obtained in 100 glomeruli.

Tubulointerstitial changes (tubular atrophy, dilation, casts, interstitial inflammation, and fibrosis) were graded as: 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 tubulointerstitial damage index (TI) in each animal was expressed as a mean value of all scores obtained.

Vascular changes were graded as: 0, no changes; 1, mild thickening of the vessel wall; 2, moderate thickening of the wall; 3, severe thickening of the wall, with ‘onion skin’ patterns; and 4, fibrinoid necrosis. Only sections of interlobular arteries and afferent arterioles were included. The resulting vascular damage index (VI) in each animal was expressed as a mean value of all scores obtained.

Statistical data were given as means ± SEM. Kruskal–Wallis and Mann–Whitney tests were used to determine the significance of the differences. The latter were considered significant when P was less than 0.05.

Results

After 8 weeks of uraemia, body weight was significantly lower in all ramipril-treated groups (Table 1). The kidney weight/body weight and left ventricular weight/body weight ratios were significantly higher in all SNx groups as compared to sham-operated controls. Systolic blood pressure in the groups treated with ramipril was significantly (P<0.05) lower compared to untreated SNx rats, irrespective of combination with HOE 140 (Table 1).

Table 2 presents the results of the morphometric investigation. GSI was significantly (P<0.05) increased in untreated SNx animals vs sham-operated controls. That increase was largely prevented in all ramipril-treated groups (P<0.05). The delayed administration of ramipril (group 6) was as effective in the prevention of glomerulosclerosis as was treatment starting immediately after SNx (group 3). In contrast, in SNx animals treated with HOE 140 alone GSI was even somewhat higher than in untreated SNx (P<0.05). No difference in GSI was found between treatment with ramipril alone or with ramipril + HOE 140.

Apparent tubulointerstitial damage was seen in all SNx animals at the end of the study. A distinct trend toward a decrease of these changes was manifest in all ramipril-treated groups, but this was statistically significant only for the group SNx-RAM + HOE.

Only slight vascular lesions (not exceeding grade 2) were present in SNx after 8 weeks. No difference in VI was found between different treatment modalities and the untreated SNx group.

Discussion

Whether the renoprotective effect of ACE inhibitors is directly related to the inhibition of the generation of angiotensin II or the intervention with other mechanisms, is of great significance as far as the rationale for the use of the new angiotensin II type 1 (AT1) receptor antagonists is concerned. Some authors suggested that potentiation of bradykinin activity, resulting from inhibition of the kininase II activity of ACE, played a major role in the renoprotective effect of ACE inhibitors [13]. Indeed, in a short-term study Hutchison et al. [14] showed that pretreatment with the B2 kinin receptor antagonist HOE 140 substantially reduced the acute antiproteinuric effect of ACE inhibitors in passive Heymann nephritis, unlike what is seen with an AT1 receptor antagonists in the same model which failed to affect albuminuria at least in the early phase of the experiment [15]. Using the puromycin aminonucleoside nephrosis model, Tanaka et al. [16] found that acute administration of the bradykinin antagonist substantially reduced the early antiproteinuric effects of ACE inhibitors and concluded that the early phase of proteinuria is independent of ANGII while progression of glomerulosclerosis was due to endogenous ANGII actions [16]. ACE inhibitors and AT1 receptor antagonists differ with respect to the hemodynamic effect in the rat kidney. An ACE inhibitor but not an AT1 receptor antagonist caused selective efferent arteriolar dilatation and hence a decrease in glomerular capillary pressure and this effect was antagonised by a B2 bradykinin receptor antagonist in a model in which the RAS was activated [13,17]. In contrast an AT1 receptor antagonist improved glomerular filtration and this was mediated by an increase in glomerular plasma flow rate and ultrafiltration coefficient.

In contrast to the above findings, in the remnant kidney model [18] chronic administration of ACE inhibitors and AT1 receptor antagonists resulted in similar lowering of proteinuria, systemic and glomerular capillary blood pressure, and retardation of the development of glomerulosclerosis. The same was also
Renoprotective effect of ACE inhibitors and kinin activity in remnant kidney

### Table 1. Biological parameters at the end of the experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Body wt (g)</th>
<th>Kidney wt/Body wt ratio (× 10⁻²)</th>
<th>LV wt/Body wt ratio (× 10⁻²)</th>
<th>Systolic blood pressure (mmHg)</th>
<th>Serum creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated (n=9)</td>
<td>471 ± 7</td>
<td>4.02 ± 0.11†</td>
<td>2.01 ± 0.05†</td>
<td>112 ± 6</td>
<td>0.58 ± 0.02†</td>
</tr>
<tr>
<td>SNx untreated (n=8)</td>
<td>456 ± 15</td>
<td>6.07 ± 0.46*</td>
<td>2.32 ± 0.1*</td>
<td>125 ± 8</td>
<td>0.74 ± 0.02*</td>
</tr>
<tr>
<td>SNx + ramipril (n=7)</td>
<td>406 ± 17*</td>
<td>6.02 ± 0.5*</td>
<td>2.22 ± 0.06*</td>
<td>92 ± 7†</td>
<td>0.74 ± 0.02*†</td>
</tr>
<tr>
<td>SNx + ramipril + HOE 140 (n=9)</td>
<td>413 ± 12*</td>
<td>5.48 ± 0.18*</td>
<td>2.49 ± 0.09*</td>
<td>104 ± 3†</td>
<td>0.72 ± 0.01*</td>
</tr>
<tr>
<td>SNx + HOE 140 (n=9)</td>
<td>453 ± 7</td>
<td>5.75 ± 0.27*</td>
<td>2.42 ± 0.05*</td>
<td>124 ± 4</td>
<td>0.72 ± 0.02*</td>
</tr>
<tr>
<td>SNx + ramipril starting</td>
<td>409 ± 13*†</td>
<td>5.34 ± 0.2*</td>
<td>2.28 ± 0.09</td>
<td>104 ± 6</td>
<td>0.76 ± 0.02*</td>
</tr>
<tr>
<td>4 weeks after surgery (n=7)</td>
<td></td>
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</tbody>
</table>

Values are means ± SEM; SNx, subtotal nephrectomy; LV, left ventricle. *P<0.05 vs sham; †P<0.05 vs untreated SNx.

### Table 2. Renal morphometric parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>Glomerulosclerosis index</th>
<th>Tubulointerstitial damage index</th>
<th>Vascular damage index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated (n=9)</td>
<td>0.02 ± 0.01†</td>
<td>0†</td>
<td>0†</td>
</tr>
<tr>
<td>SNx untreated (n=8)</td>
<td>0.24 ± 0.04*</td>
<td>1.16 ± 0.28*</td>
<td>0.25 ± 0.06*</td>
</tr>
<tr>
<td>SNx + ramipril (n=7)</td>
<td>0.10 ± 0.02**</td>
<td>0.67 ± 0.3*</td>
<td>0.19 ± 0.17</td>
</tr>
<tr>
<td>SNx + ramipril + HOE 140 (n=9)</td>
<td>0.09 ± 0.02**</td>
<td>0.42 ± 0.07**</td>
<td>0.03 ± 0.09*</td>
</tr>
<tr>
<td>SNx + HOE 140 (n=9)</td>
<td>0.45 ± 0.08**</td>
<td>1.32 ± 0.17*</td>
<td>0.27 ± 0.08*</td>
</tr>
<tr>
<td>SNx + ramipril starting</td>
<td>0.07 ± 0.01*†</td>
<td>0.53 ± 0.1*</td>
<td>0.24 ± 0.07*</td>
</tr>
<tr>
<td>4 weeks after surgery (n=7)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM. *P<0.05 vs sham; †P<0.05 vs untreated SNx.

found in uninephrectomized fawn-hooded hypertensive rats [19], rats with streptozotocin-induced diabetes mellitus [20], and MWF/Ztm rats [21]. These data can be interpreted to indicate that the decrease in angiotensin II generation is the major mechanism accounting for the renoprotective action of ACE inhibitors in renal damage models. So far, however, experiments have not been performed to formally exclude the role of increased kinin activity in the remnant kidney.

In the present study, ACE inhibitor treatment was started immediately after subtotal nephrectomy and continued for 8 weeks. This resulted in substantial attenuation of glomerulosclerosis as has been known for a long time [1]. The renoprotective effect of delayed administration of ACE inhibitors in this model is controversial [22–25]. We have found similarly low indices of glomerulosclerosis when ramipril treatment was started postoperatively (prevention of glomerular injury) or when it was delayed to week 5 after subtotal nephrectomy (reversal of glomerular injury), as can be seen by comparison of groups 5 and 6 (Table 1). This is in agreement with previous results of Lee et al. [23] in an SNx model similar to ours.

A dose of the B2 kinin receptor antagonist HOE 140 was administered which was shown to be effective in inhibiting of vascular bradykinin action [14]. If anything, glomerulosclerosis, as assessed by measurement of glomerulosclerosis index, was even greater in animals treated with HOE 140. At any rate, the specific blockade of B2 receptors did not significantly modify the action of the ACE inhibitor ramipril. Moreover, only minor changes of tubulointerstitial and vascular structure were found, and the sensitivity of our approach was apparently not sufficient to detect effects of pharmacological intervention on these parameters.

In contrast to the study of Lafayette et al. [18], who used renal ablation by ligation of the renal artery branches, we chose surgical ablation of the renal parenchyma. Unlike the ligation model, this type of ablation does not introduce a major ischaemia-related intrarenal activation of the renin–angiotensin system [26]. Different activities of this system may account for some discrepancies in literature. At any rate, the present model with modest activation of the renin–angiotensin system should have maximized the chances to see an additional or complementary effect of the intervention with the kinin system. As in our study, no effect of coadministration of HOE 140 or its absence on the renoprotective effect of an ACE inhibitor was noted by Nakamura [27]; their preliminary data in a model of antibody-induced mesangio proliferative glomerulonephritis in uninephrectomized rats indicated that glomerular injury was mainly mediated via ANGII [27]. This observation would be in line with data of the Groningen group, who studied the adriamycin model of the nephrotic syndrome and found that proteinuria was not affected by infusion of bradykinin or HOE 140 in animals treated with an ACE inhibitor whereas long-term ANGII infusion partly restored pretreatment levels of proteinuria (De Zeeuw, personal communication).

In conclusion, while ACE inhibitors had a renoprotective action in the model of surgical ablation of the kidney, this effect was not measurably altered by...
simultaneous blockade of the B2 bradykinin receptor. The observation argues against a major role of kinin accumulation in the renoprotective action of ACE inhibitors.

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References

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