Angiotensin-converting enzyme inhibition in experimental in-situ immune complex glomerulonephritis: influence on renal function, proteinuria, and morphology

F. Thaiss¹, C. Haas², U. Helmchen³, U. Wenzel¹ and R. A. K. Stahl¹

¹University Hospital Eppendorf, Department of Internal Medicine, Division of Nephrology and Osteology, Hamburg; ²Otto-von-Guericke University Hospital, Department of Internal Medicine, Division of Nephrology, Magdeburg; and ³University Hospital Eppendorf, Department of Pathology, Hamburg, Germany

Abstract

Background. Converting enzyme inhibition (CEI) ameliorates progressive loss of function in non-immune-mediated renal diseases and experimental hypertension. Little, however, is known on the potential role of CEI in established experimental chronic glomerular immune injury. We therefore studied the effect of the CEI ramipril on renal function and morphology in a model of immune mediated glomerular injury.

Methods. The immune complex glomerulonephritis was induced in uninephrectomized rats by intrarenal perfusion with the cationized antigen followed by an intravenous application of the antibody. This disease is characterized by the development of progressive albuminuria and a nephrotic syndrome (albuminuria: immune complex glomerulonephritis 342 ± 58, control 76 ± 18mg/24 h; P < 0.001). The CEI by ramipril, dissolved in the drinking water, was given 28 weeks after induction of the disease and treatment was continued for 12 weeks.

Results. In these experiments ramipril significantly reduced albumin excretion (immune complex glomerulonephritis + CEI 109 ± 16 mg/24 h; P < 0.01) when compared with untreated nephritic rats. Ramipril, however, did not significantly change inulin clearances (immune complex glomerulonephritis 224 ± 67, immune complex glomerulonephritis + CEI 278 ± 48 μl/min/100 g bw). Glomerular structural damage expressed as glomerular damage index was significantly greater in rats with immune complex glomerulonephritis when compared with controls (immune complex glomerulonephritis 0.91 ± 0.13; control 0.60 ± 0.08; P < 0.05), but was unaffected by the treatment with the CEI (immune complex glomerulonephritis + CEI 1.02 ± 0.26; control + CEI 0.63 ± 0.11).

Conclusions. These data demonstrate that ramipril reduced albuminuria in a model of immune complex glomerulonephritis; however, it failed to alter GFR and glomerular damage index. The study suggests that ramipril ameliorates proteinuria independently of obvious glomerular histological changes. The reduction of proteinuria is, however, associated with a significant decrease in filtration fraction and therefore is at least partially haemodynamically mediated.

Key words: glomerulonephritis; angiotensin-converting enzyme; proteinuria

Introduction

A large series of studies in animal models of hypertension, diabetes mellitus and other non-immune mediated glomerular injuries demonstrate that ACE inhibitors ameliorate progressive loss of renal function. The beneficial effect of ACE inhibitors in these disease entities is assumed to be not only mediated by a reduction of arterial blood pressure but also by mechanisms which involve glomerular hypertension, glomerular hypertrophy and local mediators which might act independent of hemodynamic effects [1-4].

In addition several studies in patients with diverse immune mediated glomerular injuries demonstrate a benefit from the treatment with ACE inhibitors and show that these drugs reduce proteinuria and ameliorate the progressive reduction of glomerular filtration rate [1]. These changes appear in a series of observations independent of alterations in glomerular haemodynamic function. This has led to the assumption that ACE inhibitors might act on other mediators which may disturb the permselectivity of the filtration barrier. From these studies, however, no data are available which would suggest whether ACE inhibitors might effect glomerular damage and hypertrophy when the disease is already established. We were therefore
interested in the question of whether ACE inhibitors mediate proteinuria, renal function, and structure in an animal model of immune-mediated glomerular injury when animals were treated for 12 weeks with the ACE inhibitor ramipril, starting 28 weeks after the induction of the glomerular immune injury. In treated animals ramipril reduced albuminuria. This beneficial effect appeared independent of alterations in renal function and glomerular changes in structural lesions, which suggests that ACE inhibitors might mediate proteinuria through mechanisms which are not apparent by the evaluated parameters.

Subjects and methods

Induction of immune complex nephritis (ICGN)

Male Wistar rats (body weight 100–120 g, Charles River Wiga, Langenfelde, FRG) were nephrectomized. Two weeks after nephrectomy with free access to tap water and food (normal rat chow, Altromin, Lage, FRG) nephritis was induced in left kidneys as described [5,6]. In brief, a PE10 catheter was advanced from the femoral artery into the left kidney and the kidney was flushed with PBS followed by an intrarenal injection of 100 μg cationized human IgG. The heterologous rabbit anti-human antibody was given intravenously 10 min after the circulation of the left kidney was restored.

Albuminuria and serum albumin levels

Rats were housed in metabolic cages with free access to tap water without food for 24 h. Albuminuria was measured by nephelometry (Beckmann, München, FRG) with a rabbit anti-rat albumin antibody (Cappel Laboratories, Westchester, NY) as described [6,7]. Serum albumin levels were determined by nephelometry.

Serum lipid levels

Serum triglycerides and serum cholesterol were determined by routine laboratory tests on the day of sacrifice.

Converting enzyme inhibition

The ACE inhibitor ramipril was given in the drinking water in a concentration of 100 mg/l. The water bottles were protected from light. The used concentration of ramipril increased plasma renin activity without effects on systolic blood pressure, as has been evaluated in pilot experiments.

Systolic blood pressure

Systolic blood pressure was measured weekly by tail-cuff plethysmography in awake rats as described [8].

Plasma renin activity

Blood was collected at sacrifice in ice-cooled EDTA containers. Plasma was stored at −20°C until renin activity was determined as described [7,8].

Inulin and PAH clearances

At the end of the observation period animals were placed on heated operation tables and inulin and PAH clearances were performed as described [6,9]. In brief: A PE50 catheter was placed into the right jugular vein for constant infusion. The left ureter was catheterized with a PE10 catheter for urine collection. Blood was collected in the middle of the clearance periods from the right femoral artery. Fluid losses after surgery were replaced by PBS infusion (10 ml/kg bw of PBS over 10 min), followed by a bolus of 1% inulin and PAH solution in 0.9% saline solution (3.7 ml/kg bw during a 10-min infusion period). Constant blood levels of inulin and PAH were maintained by an infusion rate of 0.9 ml/kg bw/h. Inulin and PAH were determined in serum and urine samples as described previously according to methods published by Führer et al. and by Smith et al. [10,11].

Morphology

Kidney slices were snap frozen in precooled isopentane and stored in liquid nitrogen for immunofluorescence microscopy. Tissue sections were cut and processed for immunofluorescence as described [7]. The slides were stained with FITC-labelled anti human IgG, anti rabbit IgG, anti rat IgG and anti rat C3 (Nordic Immunocchemicals, Tilburg, Netherlands). For light-microscopy renal slices were fixed in 4% buffered formaldehyde. Sections were stained with PAS as described [7,12]. Glomerular diameters and surface of the glomerular tuft were analysed by a planimeter (Centronics; GLP II; Hudson; USA). For this purpose the circumference of glomeruli was evaluated using a planimetric approach. Glomerular damage index was analysed according to Raij et al. [13] and Olsen et al. [14]. A minimum of 20 glomeruli in each tissue section was examined in a blinded fashion and the severity of the lesion was graded from 0 to 4. Slight glomerular damage, given a score of 1, included a range of abnormalities from mild increase in mesangial matrix to segmental mesangial sclerosis and/or hyalinosis with focal adhesions, involving less than 25% of the glomerulus. Mild and moderate glomerular damage, given a score of 2 vs 3, consisted of mesangial sclerosis/hyalinosis involving 25 to 75% of the glomerulus. Severe glomerular injury, given a score of 4, was characterized by involving 75 to 100% of the glomerular tuft. The extent of the injury for each individual tissue specimen was then obtained by the addition of these scores. An injury score (damage index) was then obtained by multiplying the degree of damage by the percentage of the glomeruli with the same degree of injury. The data are expressed as relative damage per glomerulus.

For electron-microscopy kidney sections were fixed in glutaraldehyde, ultrathin sections were cut and further processed as described [12].

Experimental design

Rats were uninephrectomized as described above. Two weeks after uninephrectomy, nephritis was induced in the left kidneys. Control kidneys were perfused with PBS. Therapy with ramipril started 28 weeks after induction of nephritis. CE1 was given for 12 weeks until 40 weeks after induction of nephritis. Control animals were pair fed throughout the study. The uninephrectomized nephritic rats served as the body-weight-determining animals. Animals were housed in metabolic cages for urine collection once a week. At the end
of the observation period clearance studies were performed, and kidneys were harvested for morphology. The following groups were examined: uninephrectomized controls, uninephrectomized controls treated with ramipril, uninephrectomized nephritic rats, uninephrectomized nephritic rats treated with ramipril. In each group at least 12 animals were examined at the time of sacrifice.

Statistical analysis

Results are given as means ± SD. Statistical analysis was performed by the 2-way ANOVA test. When ANOVA test was positive, then the Mann-Whitney U test was performed for statistical comparison. The confidence interval was 95%. P values <0.05 are suggested as significant.

Results

Body and kidney weights

Body weight was not significantly different between animals examined in the different groups (Table 1). There were no significant differences in kidney weights in animals at 40 weeks (Table 1).

Systolic blood pressure

Systolic blood pressures were not different in nephritic rats when compared with non-nephritic controls (Table 1). Furthermore, ramipril did not significantly reduce systolic blood pressure (Table 1).

Plasma renin activity

There were no differences in PRA in nephritic rats when compared with non-nephritic controls (Table 1). Rats treated with ramipril, however, had significantly elevated (P at least <0.01) plasma renin levels at 40 weeks after induction of disease (Table 1).

Serum lipid levels

Serum cholesterol and serum triglyceride levels were significantly elevated (P at least <0.05) in nephritic rats when compared with controls 40 weeks after induction of disease (Table 1). CEI did not reduce serum lipids in nephritic animals (Table 1).

Albuminuria

Albuminuria in nephritic rats rapidly increased early after induction of in-situ immune complex glomerulonephritis and reached a maximum at about 180 mg/24 h 3 weeks after induction of the disease. Albuminuria then decreased to reach near normal levels at about 8 weeks after induction of glomerular immune injury. Subsequently albuminuria continuously increased in nephritic rats up to 40 weeks and reached values of about 350 mg/day (Figure 1). Ramipril significantly (P < 0.001) reduced albuminuria in nephritic rats when compared with untreated nephritic rats. CEI, however, did not reduce albuminuria when compared with the pretreatment levels. CEI also did not prevent a slight increase in albuminuria in control rats at 40 weeks (Table 1).

![Albinminuria](mg/24h)

**Fig. 1.** Albuminuria in controls and nephritic rats during the 40 weeks observation period. Albuminuria did not further increase in nephritic rats when CEI was started at 28 weeks.

<table>
<thead>
<tr>
<th>Parameters 40 weeks after induction of ICGN in rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>bw (g)</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>611 ± 86</td>
</tr>
<tr>
<td>612 ± 71</td>
</tr>
<tr>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td>125 ± 2.2</td>
</tr>
<tr>
<td>11.5 ± 2.5</td>
</tr>
<tr>
<td>87 ± 5</td>
</tr>
<tr>
<td>158 ± 37</td>
</tr>
<tr>
<td>73 ± 16</td>
</tr>
<tr>
<td>3.0 ± 0.7</td>
</tr>
<tr>
<td>25 ± 59</td>
</tr>
<tr>
<td>686 ± 81</td>
</tr>
<tr>
<td>36 ± 3</td>
</tr>
</tbody>
</table>

* P < 0.01 vs. control; # P < 0.01 vs. ICGN.

Abbreviations: bw, body weight; kw, kidney weight; SBP, systolic blood pressure; PRA, plasma renin activity; S_{Chol}, serum cholesterol level; S_{Trig}, serum triglyceride level; U_{A}, albuminuria; FAC, fractional albumin clearance; C_{In}, insulin clearance; C_{PAH}, PAH clearance; FF, filtration fraction.
Serum albumin levels

Serum albumin levels were not significantly different in nephritic rats at 40 weeks when compared with nephritic rats treated with the CEI or with controls (ICGN 3.5 ± 0.9, ICGN + CEI 3.8 ± 1.3, CON 3.8 ± 0.7 mg/100 ml; n.s.).

Inulin clearance

CEI did not change C\textsubscript{in} at 40 weeks both in controls and in nephritic rats (Table 1) and inulin clearance was not significantly decreased in nephritic animals when compared with control uninephrectomized rats (Table 1).

Fractional albumin clearance

Fractional albumin clearance was significantly higher (P < 0.001) in nephritic rats when compared with non-nephritic controls at 40 weeks after induction of nephritis (Table 1). CEI significantly reduced (P < 0.01) fractional albumin clearance in nephritic rats when compared with untreated nephritic animals (Table 1).

PAH clearance

There were no significant differences in C\textsubscript{PAH} between controls and nephritic rats (Table 1). CEI significantly increased (P < 0.05) C\textsubscript{PAH} in controls and nephritic rats (Table 1).

Filtration fraction

Filtration fraction (FF%) was not significantly different between controls and nephritic rats at 40 weeks. CEI at 40 weeks significantly reduced FF% (P < 0.05) both in controls and nephritic rats (Table 1).

Glomerular morphology

By immunofluorescence microscopy anti-human-IgG (cationized antigen) and anti-rabbit-IgG (antibody) were localized in glomeruli of nephritic rats at 40 weeks after induction of nephritis. Rat C3 could be localized in a granular pattern along the glomerular capillary wall in nephritic rats at 40 weeks. There was also a faint staining of tubular basement membranes. No rat complement C3 was detected in controls.

By light-microscopy glomerular sclerosis was present additionally with tubular interstitial fibrosis (Figure 2). Glomerular surface area significantly increased (P at least <0.05) in nephritic rats when compared with controls (Figure 3). CEI, however, did not significantly change glomerular surface area (Figure 3). Glomerular damage index in nephritic rats was significantly higher (P < 0.05) when compared with uninephrectomized controls (Figure 4). CEI did not reduce the glomerular damage index (Figure 4).

By electron-microscopy subepithelial immune deposits were seen in rats with nephritis and there were no differences by CEI.

Discussion

The purpose of this study was to evaluate the role of the ACE inhibitor ramipril on renal function and structural changes in a rat model of immune-mediated glomerular disease. Ramipril treatment started 28 weeks after induction of disease when chronic renal lesions were already established.

The animal model which we used is a chronic renal injury induced in uninephrectomized rats. This model was selected since most glomerular immune injuries in two kidney models resolve after several weeks. We have characterized this model in an earlier study [6]. The disease leads to a progressive increase in albuminuria which was associated with glomerular structural damage and eventually a reduction in renal function. This glomerular lesion therefore allows to evaluate pathomechanisms which might be involved in the mediation of the development of chronic renal disease.

Proteinuria increases within a few days, reaches maximal values within four weeks and thereafter resolves, until a steady increase in albuminuria is seen after 8 weeks. The mechanism by which albuminuria resolves and increases again is not entirely clear; however, it might include a substantial defect induced by the first lesion which leads to a progressive kidney injury. The mechanisms underlying transition from acute increase and decrease of proteinuria to late increase in proteinuria again have been studied in detail in the renal ablation model and in puromycin aminonucleoside nephrosis [3,15,16]. These studies demonstrate that glomerular hypertension is a main determinant why proteinuria reappears.

A substantial body of evidence supports the theory that angiotensin II might play an important role in the pathophysiology of chronic renal injury. Besides its haemodynamic properties, angiotensin II might regulate renal growth and hypertrophy [17–19]. Most of this evidence is derived from studies which applied ACE inhibitors or AII blockers [16,20–22].

The ACE inhibitor used in the present experiments was ramipril. Ramipril is completely absorbed after oral ingestion and effectively inhibits ACE activity. Ramipril is known to have a longer half-elimination time than captopril [23,24]. The dose used (100 mg/l drinking water) was chosen because our pilot studies (see Results) demonstrated that the dose used did not significantly reduce blood pressure, although plasma renin activity was increased at least threefold.

In nephritic rats treated with ramipril albuminuria was significantly decreased when compared with untreated nephritic rats. CEI abolished the further increase in albuminuria in nephritic rats; it did, however, not reduce albuminuria when compared with the pretreatment level at 28 weeks after induction of the disease. This reduction in albuminuria in treated versus untreated nephritic rats was associated with increased PAH clearances. Inulin clearances, however, were not influenced by CEI. Thus reduced albuminuria was associated with a reduction in filtration fraction.
Fig. 2. Light-microscopy of kidneys at the end of the 40 weeks observation period. (a) Renal cortex with normal glomeruli, tubules and interstitium (untreated control) (PAS staining, magnification x90). (b) Renal cortex with partially scarred glomeruli, focal tubular and interstitial lesions (untreated nephritic animal) (PAS staining, magnification x90). (c) Normal glomerulus (untreated control) (PAS staining, magnification x720). (d) Chronic damage with scarred glomerular tuft and adhesions to Bowman's capsule 40 weeks after induction of glomerular immune injury (untreated nephritic animal) (PAS staining, magnification x720).

Therefore our results demonstrate that albuminuria in nephritic rats might be reduced mainly due to haemodynamic changes following CEI.

The role which CEI might play in proteinuria and haemodynamic alterations in experimental glomerular injury was examined in detail in the renal ablation model and in aging rats [4,16,21,22]. These studies demonstrate that haemodynamic changes after renal ablation mediate both proteinuria and morphology. More recently, however, a couple of studies have shown that morphological changes in the renal ablation model may be induced by structural hypertrophy and are not simply related to haemodynamic changes [25–29]. We therefore examined whether the ACE inhibitor additionally might effect kidney weight and morphology in rats with chronic immune complex disease. In our studies CEI did not reduce kidney weight in nephritic rats at 40 weeks when compared
ACE inhibition in immune complex GN

Fig. 3. Glomerular surface area at 40 weeks after induction of nephritis. Glomerular surface area was significantly (P<0.01) greater in nephritic rats when compared with controls. CEI did not reduce glomerular surface area.

Fig. 4. Glomerular sclerosis index in controls and nephritic rats at 40 weeks. Sclerosis index was significantly (P<0.01) greater in nephritic rats when compared with controls at 40 weeks. CEI did not change sclerosis index.

with untreated animals. In addition, glomerular surface area and glomerular damage index were not reduced by CEI in nephritic rats. Glomerular surface area and damage index were, however, significantly greater in nephritic rats when compared with uninephrectomized controls. This negative finding with respect to glomerular morphology in our studies is not due to dose schedule and the time period studied. The dose of CEI was sufficient, as Ikoma et al. used 200 mg/l enalapril as a 'high dose' regimen [30]. Thus glomerular damage index was not improved by ACE inhibitor therapy.

Another possibility may be considered as to why glomerular morphology was not improved by CEI. In our studies serum lipid levels were not reduced by CEI. A number of studies have demonstrated that increased lipid levels may aggravate glomerular lesions [12,35,36]. Hypercholesterolaemia may also augment glomerular and tubulointerstitial macrophage infiltration and the inflammatory burst released by these cells [37,38].

Clinical trials have emerged which demonstrate that CEI decreased progression of renal insufficiency [39] and has a greater antiproteinuric effect for a given decrease in blood pressure [40]. In view of the available experimental data with the well-characterized effects of CEI, the decrease in glomerular transmembranous pressure, arrest of glomerular growth, improved glomerular permeability, activation of bradykinin, and the possible additional beneficial effects of excess doses of ACE inhibitors; much, however, remains to be learned about the role which CEI might play in therapy of glomerulopathies [41].

Our study demonstrates that reduced albuminuria and fractional albumin clearance by CEI not necessarily reflects improved glomerular morphology. This raises the question of whether repeated kidney biopsies in patients treated with CEI might become necessary to demonstrate convincingly the effect of CEI on glomerular morphology.

Acknowledgements. Supported by the Deutsche Forschungsgemeinschaft (DFG Th 343/1-2 and Sta 193/6-2). The authors thank Dr U. Pooth, Frankfurt, FRG, for supply of the ACE inhibitor ramipril.

References
3. Anderson S, Rennke HG, Brenner BM. Therapeutic advantage of converting enzyme inhibitors in arresting progressive renal...

Received for publication: 26.6.94
Accepted in revised form: 27.7.95