Proteinuria-associated renal injury and the effects of intervention in the renin-angiotensin-aldosterone system
Kramer, Andrea Brechtsje

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Chapter 2

Inter-individual differences in antiproteinuric response to ACEi in established adriamycin nephrotic rats are predicted by pretreatment renal damage

Andrea Kramer, Goos Laverman, Harry van Goor and Gerjan Navis

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ABSTRACT

ACE inhibition (ACEi) reduces proteinuria and provides renoprotection, but not all subjects benefit from ACEi. Individual differences in reduction in proteinuria at onset of treatment, and in residual proteinuria during therapy predict differences in renal outcome. In this study, we investigated whether individual differences in antiproteinuric efficacy of ACEi are explained by differences in severity of pretreatment renal structural damage. Secondly, whether differences in the level of residual proteinuria during therapy are explained by the severity of renal structural damage at that time, in adriamycin nephrosis in the rat. Pretreatment renal structural damage was assessed in biopsies 6 weeks after adriamycin (2 mg/kg i.v.). Then, ACEi (lisinopril 75 mg/l, n=23) or vehicle (n=10) were started; renal biopsies were repeated after stabilization of antiproteinuric response (week 8). Early renal damage (interstitial α-smooth muscle actin expression and macrophage accumulation) and established lesions (focal glomerulosclerosis (FGS) and interstitial fibrosis) were scored. During ACEi, proteinuria fell from 834 (487-851) mg/24h pretreatment to 153 (66-265) at week 8 (p<0.05); FGS stabilized from 27 (4-20) pretreatment to 26 (4-84) at week 12, whereas vehicle did not affect proteinuria, resulting in progressive FGS: 18 (10-26) versus 88 (46-130) (p<0.05). All parameters of pretreatment damage significantly predicted antiproteinuric response. Residual proteinuria during ACEi significantly correlated to renal structural damage parameters at that time. Pretreatment renal damage also predicted renal outcome during extended treatment. Thus, in this experimental setting, in rats with the same renal disorder and same duration of disease, individual differences in pretreatment renal damage, albeit relatively modest, explain individual differences in renal responsiveness to ACEi. This implicates that the limits of the efficacy of ACEi are set by prevalent renal damage. Further studies into the mechanisms of individual resistance to the antiproteinuric action of ACEi are needed to develop additive intervention strategies.
INTRODUCTION

Angiotensin-converting enzyme inhibitors (ACEi) provide renoprotection in man and in experimental renal disease, by their effects on blood pressure, renal haemodynamics, and proteinuria [1-3]. The reduction in proteinuria appears a pre-requisite for long-term renoprotection [4]. However, individual differences in antiproteinuric effect are large, with residual proteinuria in many patients. These differences are important, as both the fall in proteinuria at onset of therapy, and the severity of residual proteinuria after stabilization of therapy response predict long-term renal prognosis [5-10]. This predictive effect is considered to support the causal role of proteinuria reduction in renoprotection, but, on the other hand, has also been interpreted to identify individuals deemed to progressive renal damage due to the nature or severity of their renal disorder.

Individual differences in severity of renal damage before start of therapy may well play a role in differences in antiproteinuric effect of ACEi, as suggested by retrospective data in man [11] and by the limited efficacy of ACEi in advanced versus early renal disease in several experimental models [12;13]. However, the predictive value of individual differences in severity of pretreatment renal damage for the subsequent antiproteinuric response to ACEi has not been tested prospectively. Therefore, we prospectively investigated the predictive value of individual differences in renal structural damage for individual differences in antiproteinuric effect of ACEi in established adriamycin nephrosis, a well-validated, normotensive model of proteinuria-induced renal damage [7]. We investigated, first, whether pretreatment renal damage predicts the reduction in proteinuria 2 weeks after start of ACEi treatment, and second, whether the level of residual proteinuria after stabilization of the therapy response reflects the severity of renal structural damage at that time [14;15].

METHODS

Experimental groups

Fifty-seven male Wistar rats weighing 282±9 g (Cpb. Wu; Harlan, Horst, the Netherlands) were housed in a temperature- and light-controlled room with free access to food and water. To obtain optimal therapeutic efficacy of ACEi, all rats received a low sodium diet [7] (0.05% NaCl, 20% protein, Hope Farms Inc., Woerden, The Netherlands) starting 2 weeks prior to induction of nephrosis [15]. Nephrosis was induced in 49 rats by injecting 2 mg/kg adriamycin into the tail vein under anaesthesia. The 8 remaining rats served as healthy controls (CON). After stabilization of proteinuria at 6 weeks, animals were stratified for proteinuria, rearranged in 4 groups and instituted on active treatment with the ACEi lisinopril [7] or vehicle for 6 additional weeks, that is until termination. Renal biopsy procedures were performed at 6 and 8 weeks, the latter because 2 weeks after start of therapy residual proteinuria is stabilized [7;15]. The following groups were studied:
ACEi group (ACEi, n=23). Treatment with lisinopril (75 mg/l drinking water) from week 6 until week 12. Renal biopsies at week 6 and 8.


ACEi (n=10) biopsy controls. These rats only underwent the biopsy at week 8 to test whether the pretreatment biopsy affected the early antiproteinuric response or the extent of renal damage. Vehicle biopsy controls (n=6). No biopsies were performed on these animals to test the possible impact of biopsies on the natural course. Biopsies did not affect the outcome on FGS and interstitial fibrosis (data not shown) in the ACEi and VEH groups compared to the biopsy control groups. Body weight (BW) and the food and water intake were measured weekly. Urinary protein excretion was measured by the Biuret method (Bioquant\textsuperscript{TM}, Merck, Darmstadt, Germany). Plasma and urine creatinine levels were determined colorimetrically (Sigma Chemical Co, St. Louis, MO, USA). Systolic blood pressure (SBP) was measured by the tail cuff method in trained conscious rats [16]. At the end of the study at week 12, kidneys were removed and processed for histologic examination [17] and animals were sacrificed. All operations took place under Isoflurane/O\textsubscript{2}/N\textsubscript{2}O anaesthesia. Renal biopsy procedures were performed via a dorsolateral incision. Immediately after surgical removal of a small part of the renal lower pole, gelfoam (Spongostan\textsuperscript{®}) was applied to reach haemostasis. The first renal biopsy was obtained from the left kidney, the second from the right kidney. All procedures were approved by the Committee for Animal Experiments of the University of Groningen, the Netherlands.

Tissue processing and (immunohistochemical) staining procedures

Renal tissue was fixed in 4% paraformaldehyde and processed for paraffin embedding. Paraffin sections (4\textmu m) were stained with periodic acid-Schiff (PAS) to evaluate glomerular and interstitial damage. For staining procedures, paraffin sections were dewaxed and subjected to heat induced antigen retrieval by overnight incubation in 0.1 M Tris/HCl buffer on 80°C. Endogenous peroxidase was blocked with 0.075% H\textsubscript{2}O\textsubscript{2} in phosphate-buffered saline (PBS) for 30 min. Alpha-smooth muscle cell actin (\(\alpha\)-SMA) was detected using a murine monoclonal antibody (clone 1A4, Sigma Chemical Co., St. Louis, MO, USA) for 60 min. Macrophages were detected using ED1 antibody (Serotec Ltd, Oxford, UK). Binding for both antibodies was detected using sequential incubations with peroxidase labelled rabbit anti-mouse and peroxidase-labelled goat anti-rabbit antibody (Dakopatts, DAKO, Glostrup, Denmark) for 30 min. Peroxidase activity was developed using 3,3'-diaminobenzidine tetrachloride (DAB) for 10 min.

Renal morphology

Focal glomerulosclerosis (FGS), defined as glomerular areas with mesangial expansion and adhesion formation simultaneously present in one segment, was scored semi-quantitatively on a scale 0 to 4 [17;18]. Interstitial damage was scored semi-quantitatively on a scale 0 to 3 [17]. Alpha-SMA staining and interstitial ED1 positive cells were measured using computer-assisted
morphometry. Twenty cortical interstitial images without vessels or glomeruli were selected and the total immunohistochemical-staining surface for α-SMA was measured and divided by the total surface of the image. To measure interstitial ED1 positive cells, 30 cortical interstitial fields, excluding glomeruli and large arteries, were measured. The computer estimates the number of cells by counting the stained dots per field. The number of glomerular ED1 positive stained cells was determined by manual counting of 25 glomeruli.

Statistical analyses

Results are expressed as median and 95% confidence interval of the median, calculated according to the table: Ranks for obtaining confidence interval for the median [19]. Statistical analysis of group differences was performed by a Kruskal-Wallis ANOVA on ranks. Intra-individual differences between week 6, 8 and 12 were analysed by using the paired Students T-test in case of normal distribution of data; otherwise by using the Wilcoxon signed rank test. For pre- and post-treatment values of SBP and proteinuria we used the values of week 6 respectively week 12. Spearman correlation coefficients were calculated. Antiproteinuric therapy response was analysed in a dual way. First, with the % reduction in proteinuria between week 6 and 8 as dependent variable, and with pretreatment markers of renal structural damage (α-SMA, macrophage influx and FGS) as independent variables. This analysis cannot be performed for the pretreatment proteinuria because of the involvement of pretreatment proteinuria in the % reduction between week 6 and 8. Therefore we analysed using the residual proteinuria at week 8 as antiproteinuric therapy response. For the long-term outcome the FGS score at week 12 was entered as dependent variable with markers of structural damage at week 6 and 8 as independent variables. Multiple regression analysis was performed with the markers of renal damage at week 6 and 8 to test if markers at week 8 had more explanatory power for the long-term outcome. For regression lines between FGS week 6 and 12, regression analysis was used. All statistical analyses were calculated using SPSS statistical software version 10.0. Statistical significance was assumed at the 5% level.

RESULTS

Group data: clinico-pathologic parameters

Group characteristics are shown in Table 1. Throughout the experiment food and water intake was similar in all groups, consistent with a good condition of the nephrotic animals. Body weight was significantly lower in all adriamycin groups compared to control rats (p<0.01 for week 6, 8 and 12), and no differences between the adriamycin groups were observed. In the ACEi group one animal died. In the VEH group four animals died.

ACEi significantly reduced proteinuria at group level (Figure 1), whereas proteinuria remained unchanged in the VEH groups. During the first 2 weeks of therapy the reduction in proteinuria was steep, with a stable residual proteinuria as of week 8. The time course of
proteinuria was not affected by the biopsy procedures, neither in the ACEi-treated animals, nor in the VEH treated animals. Creatinine clearance was impaired in the adriamycin rats compared to healthy controls (p<0.05). Systolic BP was significantly reduced by ACEi therapy compared to pretreatment values (p<0.01) and compared to VEH (p<0.05).

**Figure 1.** Proteinuria time course. Values in ACEi treated animals decreased as result of treatment. Values in untreated animals remained stable after wk 6.

*p<0.05 vs VEH

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**Table 1.** Group characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>ACEi</th>
<th>VEH</th>
<th>CON</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(n=22)</td>
<td>(n=6)</td>
<td>(n=8)</td>
</tr>
<tr>
<td>Body weight (gram)</td>
<td>wk 6</td>
<td>400 (390-414) b</td>
<td>400(362-420) b</td>
<td>453 (408-490)</td>
</tr>
<tr>
<td></td>
<td>wk 8</td>
<td>396 (372-406) b</td>
<td>380 (358-416) b</td>
<td>479 (440-536)</td>
</tr>
<tr>
<td></td>
<td>wk 12</td>
<td>396 (364-430) b</td>
<td>388 (374-410) b</td>
<td>495 (414-550)</td>
</tr>
<tr>
<td>Proteinuria (mg/24h)</td>
<td>wk 6</td>
<td>834 (487-851) b</td>
<td>777 (594-960) b</td>
<td>51 (26-100)</td>
</tr>
<tr>
<td></td>
<td>wk 8</td>
<td>153 (66-265) c</td>
<td>697 (531-863)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>wk 12</td>
<td>117 (75-268) a-d</td>
<td>877(625-1129) b</td>
<td>53 (26-128)</td>
</tr>
<tr>
<td>Creatinine Clearance (ml/min)</td>
<td>wk 12</td>
<td>1.28(1.03-1.51) b</td>
<td>1.19(0.65-2.80) b</td>
<td>2.15(1.73-4.20)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>wk 6</td>
<td>145 (141-151)</td>
<td>148 (140-154)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>wk 8</td>
<td>99 (89-116) c-d</td>
<td>144 (97-156)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>wk 12</td>
<td>104 (90-116) c-d</td>
<td>139 (102-177)</td>
<td></td>
</tr>
</tbody>
</table>

*Results are given as median and 95% CI.  b P<0.01 vs CON,  c P<0.05 vs wk 6,  d P<0.05 vs VEH

**Group data: structural changes (Table 2)**

Pretreatment FGS score was low in all groups. A significant rise in FGS score from week 6 until endpoint was observed in the VEH group only (p<0.05), whereas in the treated groups FGS remained stable (Figure 2A-D). Consequently, at termination FGS score was significantly lower in the ACEi treated animals. Pretreatment scores for interstitial fibrosis (IF) were increased...
in the adriamycin groups at week 6 as compared to healthy controls (p<0.05). ACEi treatment had no effect on IF.

In the adriamycin groups, but not in healthy controls (p<0.05), α-SMA was expressed in interstitial myofibroblasts. Some degree of expression was observed in the glomerular mesangial areas, but in interstitial parts with atrophied tubuli (Figure 2E and F) expression was abundant. The α-SMA score was not affected by treatment.

Interstitial macrophage influx was predominantly seen around tubuli, glomeruli and arteries (Figure 2G and H). Although glomerular macrophages were present, no differences were found between adriamycin and controls (data not shown). Pretreatment interstitial macrophage accumulation was increased (p<0.01 adriamycin versus controls) in all groups. After 2 weeks of therapy, interstitial macrophage accumulation was significantly (p<0.05) reduced in treated groups compared to pretreatment, but not in VEH. At endpoint, macrophage accumulation was significantly decreased versus week 6 in all groups (p<0.05).

All pretreatment parameters of renal damage were significantly and positively correlated to proteinuria at week 6, with r-values of 0.82; 0.73; 0.77 for FGS, α-SMA and macrophages respectively, all p<0.01

Table 2. Results of semi-quantitative and morphometrical analysis of structural damage

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>ACEi (n=22)</th>
<th>VEH (n=6)</th>
<th>CON (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal glomerulosclerosis</td>
<td>wk 6</td>
<td>27 (4.70)</td>
<td>18 (10.26)</td>
<td></td>
</tr>
<tr>
<td>(scale 0-400)</td>
<td>wk 8</td>
<td>30 (6.44)</td>
<td>30 (16.44)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>wk 12</td>
<td>26 (4.84)</td>
<td>88 (46-130)</td>
<td></td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>wk 6</td>
<td>0.5 (0-2)</td>
<td>0.0 (0-2)</td>
<td></td>
</tr>
<tr>
<td>(IF, scale 0-4)</td>
<td>wk 8</td>
<td>1.0 (0-2)</td>
<td>1.0 (0.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>wk 12</td>
<td>1.0 (1-2)</td>
<td>0.0 (0-2)</td>
<td></td>
</tr>
<tr>
<td>α-SMA staining</td>
<td>wk 6</td>
<td>7.5 (5.6-8.4)</td>
<td>7.7 (4-11)</td>
<td></td>
</tr>
<tr>
<td>(% per interstitial field)</td>
<td>wk 8</td>
<td>8.5 (5.8-10.3)</td>
<td>9.4 (4.5-12.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>wk 12</td>
<td>5.5 (4.4-8.5)</td>
<td>9.6 (3.7-16.1)</td>
<td></td>
</tr>
<tr>
<td>Interstitial macrophages</td>
<td>wk 6</td>
<td>88 (36-138)</td>
<td>73 (49-321)</td>
<td></td>
</tr>
<tr>
<td>(number per interstitial field)</td>
<td>wk 8</td>
<td>37 (33-87)</td>
<td>78 (8-109)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>wk 12</td>
<td>13 (7-18)</td>
<td>9 (2-44)</td>
<td></td>
</tr>
</tbody>
</table>

Data expressed as median and 95 % CI. a P<0.05 vs CON, b P<0.05 vs wk 6, c P<0.05 vs ACEi

Individual data: renal predictors of antiproteinuric response

The % change in proteinuria by ACEi was significantly predicted by all tested pretreatment parameters of renal structural damage (α-SMA, macrophage influx and FGS), with less reduction in proteinuria for higher pretreatment damage scores (shown for % change week 6-8 in Table 3). Similarly, residual proteinuria during ACEi was predicted by all pretreatment
parameters of renal damage (Table 3). Pretreatment proteinuria was positively correlated to the % change in proteinuria during ACEi (r=0.58, p<0.01) and to residual proteinuria during ACEi (week 8: r=0.85, p<0.01). However, since these parameters are arithmetically related no predictive value should be attributed to these correlations. No predictors could be identified for blood pressure response.

**Individual data: renal correlates of residual proteinuria at stabilization of response.**

To investigate whether the level of residual proteinuria was explained by the extent of renal structural damage at time of stabilization of antiproteinuric response, correlation coefficients were calculated for proteinuria at week 8 with the parameters of renal structural damage at week 8 in ACEi-treated rats. All parameters of renal damage were significantly and positively correlated with residual proteinuria at that time, with r-values of 0.77 for FGS, 0.46 for α-SMA and 0.53 for macrophages, (all p<0.05), respectively.

**Table 3.** Pretreatment markers of structural renal damage versus short and long-term therapy response

<table>
<thead>
<tr>
<th>Short-term</th>
<th>Long-term (FGS wk 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(% Δ UP wk 6-8)</td>
</tr>
<tr>
<td><strong>ACEi</strong></td>
<td><strong>ACEi</strong></td>
</tr>
<tr>
<td>FGS wk 6</td>
<td>0.56*</td>
</tr>
<tr>
<td>α-SMA wk 6</td>
<td>0.60*</td>
</tr>
<tr>
<td>mø wk 6</td>
<td>0.69*</td>
</tr>
</tbody>
</table>

All values are r-values (Spearman correlation coefficients); UP, proteinuria; α-SMA, α-smooth muscle cell actin; FGS, focal glomerulosclerosis; mø, macrophages * p<0.01, # p<0.05

**Individual data: renal predictors of therapy response during extended treatment.**

Finally, we evaluated whether the predictive effect of pretreatment damage in this study persisted during extension of treatment up to week 12 (Table 3). For FGS at week 12, on univariate analysis, the pretreatment values of α-SMA, macrophages and FGS were all strongly predictive in the ACEi group, being worse in animals with the highest scores for pretreatment renal damage. Pretreatment proteinuria, as well as the early % reduction in proteinuria (week 6-8) also predicted FGS at week 12 (r=0.87 and 0.69, both p<0.01 for the ACEi group).

**Figure 2**

Representative photomicrographs showing glomerular and interstitial injury in (A) a healthy control, (B) an ADR treated rat at week 6 (pretreatment damage), (C) an ACEi treated rat, and (D) a VEH rat. PAS staining. α-SMA staining in a healthy control (E) (in blood vessel walls, arrowheads) and in an ADR-treated rat (F) at week 6 showing expansion of fibrotic structures (arrowheads). ED-1 staining for macrophages in an ADR treated rat at week 6 (G) and at week 12 (H).
Figure 2

Renal damage determines antiproteinuric effect
Similar predictive values of pretreatment renal damage were found for other renal parameters at week 12, i.e. creatinine clearance and interstitial α-SMA expression (data not shown).

The values of α-SMA, macrophages and FGS after 2 weeks of treatment were also positively correlated with the long-term outcome on univariate analysis. However, on multiple regression analysis the values at week 8 did not provide additional explanatory power over the pretreatment values.

Thus, the relationship between pretreatment renal damage and renal prognosis is preserved during intervention, despite the significant protective effect of treatment. This is illustrated in Figure 3: these correlation plots between pretreatment FGS and FGS at termination in untreated and treated rats demonstrate that active treatment shifts the relationship towards a more favourable outcome.

**DISCUSSION**

As anticipated, ACEi reduced proteinuria and stabilized renal structural damage, with considerable individual differences. Our data show that individual differences in extent of pretreatment renal damage consistently predicted the antiproteinuric efficacy of ACEi. This is apparent from, first, the predictive value of pretreatment renal parameters for the subsequent fall in proteinuria by ACEi, and second, the close correlation between the parameters of renal damage and residual proteinuria after stabilization of therapy response.

ACEi generally are effective antiproteinuric agents at group level, but individual differences in antiproteinuric effect are large. In a post-hoc study in man we have found that antiproteinuric responsiveness is an individual characteristic, that is only slightly modulated by differences in dose or class of drug [20]. Our present study extends on those data, demonstrating that it is the severity of prior renal damage that limits the antiproteinuric efficacy of ACEi.
Renal damage determines antiproteinuric effect

This was analysed in a dual fashion, that is for the change in proteinuria from baseline, and for residual proteinuria after stabilization of therapy response, as both parameters are well-established, non-invasive predictors of long-term renal prognosis [5-7]. The results of the two analyses were strongly concordant; and in fact these two analyses should probably be considered as different angles to analyse the same phenomenon, rather than as separate findings. Their close correspondence enhances the robustness of the data.

In this study, all parameters of renal damage were closely interrelated at all points in time, and were similarly predictive for therapy response. Considering these interrelationships, our data do not allow to dissociate between the separate indices of renal damage for their predictive value. However, their consistency provides additional support for the conclusion that individual differences in underlying renal damage account for individual differences in responsiveness to antiproteinuric intervention.

Our analysis primarily focussed on antiproteinuric response, but also allows some inferences on other renal outcome parameters. Within the limited time frame of our study, at group level ACEi provided significant protection against FGS, but did not affect the pre-fibrotic changes in the interstitium or creatinine clearance. For the purpose of the present study, the relevant finding is that the individual predictive value held true for all renal outcome parameters, irrespective of whether or not ACEi exerted a significant effect at group level. Longer follow-up would be needed to assess the predictive value of pretreatment renal damage for eventual protection against end-stage renal damage.

No predictive effect of the renal lesions on the antihypertensive effect of intervention was found. It is questionable, however, whether in this normotensive model, reduction in blood pressure provides a good index of the sensitivity to renoprotective intervention.

Many factors can affect the efficacy of ACEi, such as sodium intake, protein intake, differences in underlying renal disorder, and early versus late start of therapy [12;21;22]. As the purpose of our study was to dissect the role of individual differences in renal damage, we eliminated these sources of variability as much as possible, by studying a single, well-defined disease condition, starting therapy at a fixed point in time. Our finding that pretreatment renal structural damage limits antiproteinuric efficacy is in accord with other experimental studies of different design, and in other models. In subtotally nephrectomized rats, ACEi stabilized glomerular structure in non-sclerotic or early-sclerotic glomeruli, but not in glomeruli with higher sclerosis indices before onset of treatment [13]. However, no data on antiproteinuric efficacy were provided. Starting ACEi before renal ablation (that is before presence of renal damage) could largely prevent subsequent glomerular injury, whereas only partial protection was obtained when ACEi was started 8 weeks after ablation [23], a finding corroborated by a more recent study [24]. In 5/6 nephrectomy as well as in passive Heymann nephritis early treatment with ACEi reduced proteinuria and the resulting markers of interstitial inflammation, whereas in the advanced phase of disease ACEi failed to exert these effects [12].
We started ACEi 6 weeks after disease induction. At that time on morphological examination the severity of renal damage was still relatively mild. Thus, the poor antiproteinuric effect of ACEi in animals with the highest scores for renal damage at week 6 cannot be attributed to presence of advanced renal lesions at that time. If not due to extensive renal lesions, what factors could influence antiproteinuric response? In our study the early renal lesions, as apparent from α-SMA expression, and structural damage, as apparent from FGS, were not reversible. Their association with a poor reduction of proteinuria might therefore be a marker of the contribution of irreversible lesions in the total proteinuria.

ACE inhibition during nephrosis probably exerts the therapeutic effect by different mechanisms – at least by three. First, the inhibition of the renin-angiotensin system (RAS) pathway results in altered intra-glomerular and intra-renal haemodynamics [4]. Second, inhibition of the production of angiotensin II results in a reduced bio-activation of TGF-β and subsequent reduction of interstitial accumulation of extracellular matrix [25]. Finally, in rats with adriamycin nephrosis ACE inhibition results in a preservation of heparan sulphate proteoglycans, which are thought to play a crucial role in perm-selective properties of the glomerular basement membrane [26]. It would be of interest to know which of these mechanisms might be adversely affected by the presence of renal structural abnormalities as observed here, but our study was not designed to address this issue. The only conclusion we can make in this respect is that the response of systemic blood pressure was unaltered.

We used an ACEi regimen well established to exert the maximum benefit in this setting, that is ACEi dose on the top of the dose response for proteinuria, and a low sodium intake. We recently reported that, in this setting, addition of angiotensin II type 1 receptor blockade does not overcome the individual therapy resistance to ACEi [27]. Taken together with the present data, this implies that a poor antiproteinuric response identifies individuals in whom adequate renoprotection cannot be obtained by RAS-blockade alone, and will require other, or combined modes of intervention. ACEi only partly protects against interstitial inflammation and fibrosis [28]. Co-treatment with mycophenolate mofetil (MMF) or a statin, which both do not exert renoprotection as a monotherapy, potentiates the renoprotective action of ACEi [24;29;30], suggesting that MMF and statin therapy might interfere with specific pathways of resistance to ACEi in these models. Whether this can be applied to overcome individual resistance to RAS-blockade remains to be determined.

What are the clinical implications of our findings? In man, established renal damage is usually present by the time patients come to medical attention. Our data suggest that the limits of therapeutic benefit of ACEi may already be set at that time, and [6;31] that individuals in whom RAS-blockade alone will not provide sufficient renoprotection can be identified early in the course of treatment [20]. Whereas this assumption seems to be supported by retrospective data in renal transplant recipients, in whom renal interstitial lesions predicted the antiproteinuric response to ACEi [11], it needs further prospective confirmation. Moreover, the relative impact
Renal damage determines antiproteinuric effect

versus other modifiers of therapy response (disease condition, sodium and protein intake) is important to consider.

In conclusion, pretreatment renal damage limits the antiproteinuric efficacy of pharmacological intervention by a standardised ACEi regimen in this model of proteinuria-induced renal damage. Subjects requiring additional modes of intervention to obtain effective long-term renoprotection can be identified early in the course of treatment. Further studies will have to elucidate the specific pathways involved in the therapy resistance in these individuals.

References


