Do severe systemic sequelae of proteinuria modulate the antiproteinuric response to chronic ACE inhibition?

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Abstract

**Background.** ACE inhibition exerts an antiproteinuric and renoprotective effect. However, residual proteinuria is often present. As residual proteinuria is associated with a poor renal outcome, identification of its determinants is important. We found previously that the systemic sequelae of proteinuria enhance renal damage in untreated nephrotic rats. The impact of systemic nephrosis on renal therapy response, however, is unclear. In the present study we therefore investigated whether the severity of systemic nephrosis, estimated from plasma cholesterol, predicts residual proteinuria during ACE inhibition.

**Methods.** Sixty male Wistar rats with established adriamycin nephrosis were studied. Six weeks after the induction of nephrosis, rats were stratified for proteinuria and treated for 2 weeks with lisinopril (75 mg ul) or vehicle.

**Results.** At the start of treatment, median proteinuria was 744 mg day (95% confidence interval (CI) 609–860) and plasma cholesterol was 10.4 mmol l (95% CI 8.0–12.6), reflecting the state of systemic nephrosis. Lisinopril, but not vehicle, reduced blood pressure and proteinuria (–62%; range –70 to –48; P = 0.001). Residual proteinuria was 275 mg day, with a wide range (47–1119 mg day). Pre-treatment proteinuria and pre-treatment cholesterol correlated positively with residual proteinuria. By multivariate analysis (r² of model = 0.92), both pre-treatment cholesterol and pre-treatment proteinuria were independent predictors of residual proteinuria. The quantitative impact of this multivariate analysis is illustrated by the difference in residual proteinuria between rats with a cholesterol:proteinuria ratio less than, compared with greater than, the median (residual proteinuria 298 mg day (CI 129–496) vs 439 mg day (CI 158–670), respectively). Blood pressure response was not predicted by the tested predictor variables.

**Conclusions.** In this model of proteinuria-induced renal damage, not only proteinuria as such, but also the concomitant nephrotic alterations predict residual proteinuria. Further studies, applying specific interventions, are needed to determine which components of the systemic derangements could play a causal role in the modulation of therapy response.

**Keywords:** established adiriamycin nephrosis; lisinopril; plasma cholesterol; proteinuria; residual proteinuria; systemic nephrosis

Introduction

It is well established that ACE inhibition exerts an antiproteinuric and renoprotective effect in experimental as well as human renal disease [1–3]. Residual proteinuria, however, is often present and is associated with a poor long-term renal prognosis [1,4]. Identification of the factors involved in therapy resistance, therefore, is important as this may guide the development of additional treatment strategies to optimise individual antiproteinuric treatment in subjects with a poor ACE-inhibition response.

A factor that might contribute to therapy resistance in proteinuric renal disease is the severity of the metabolic derangements elicited by proteinuria, i.e. the state of ‘systemic nephrosis’. Proteinuria elicits a complex state of metabolic derangements, including sodium retention, volume expansion, hypoalbuminaemia, coagulation abnormalities and dyslipidaemia. We demonstrated recently that not only proteinuria, but also the severity of the systemic derangements, provides an independent contribution to the progression of renal damage in untreated adiriamycin-nephrotic rats [5]. Whether the severity of the systemic derangements could also modify therapy response, however,
has so far not been investigated, neither in man nor in experimental animals.

In the present study, therefore, we investigated whether the severity of the systemic alterations elicited by proteinuria—as estimated from plasma cholesterol—could predict the response to chronic ACE-inhibition in established adriamycin nephrosis. This rat model of proteinuria-induced renal damage is characterized by nephrotic range proteinuria, accompanied by the typical systemic nephrotic alterations. Hyperlipidaemia, with an elevated total plasma cholesterol, and hypoalbuminaemia are present, and both are in proportion to proteinuria [1,5,6]. For the present analysis, we used cholesterol as a marker for the severity of this complex metabolic state.

Methods

The protocol was approved by the Committee for Animal Experiments of the University of Groningen, The Netherlands. In this study, 60 male Wistar rats (Hsd.Cpd.Wu; Harlan Inc., Zeist, The Netherlands) were studied. Throughout the study the rats were housed in a temperature-controlled room with a 12-h light–dark cycle. All animals were fed a low sodium diet (0.05% sodium, 20% protein; Hope Farms Inc., Woerden, The Netherlands) ad libitum from 1 week before study entry and received daily fresh tap water ad libitum. To determine proteinuria, urine was collected once a week throughout the entire protocol during a 24-h stay in metabolic cages with free access to food and water. Systolic blood pressure was measured weekly in conscious rats by tail-cuff with an automated multichannel system (Apollo 179; IITC Life Science, Woodland Hills, CA, USA). During each session, two measurements were recorded for each animal. The values were taken as the mean of these two recordings. Animals were trained before study entry to become accustomed to handling, metabolic cages and blood pressure measurements.

Induction of nephrosis

Rats were anaesthetized with Isoflurane/O₂:N₂O and 1.5 mg/kg adriamycin was injected in the penis vein. This dose of adriamycin was chosen to induce a modest and stable proteinuria, as confirmed by previous experiments in our laboratory by Wapstra et al. [7] and later modified by De Boer et al. [5] in connection with a different rat strain. In this model, proteinuria stabilizes 6 weeks after injection of adriamycin in the nephrotic range. Cholesterol is elevated and albumin decreased in proportion to total urinary protein loss [1,5]. Cholesterol values are closely and negatively correlated to albumin values, with correlation coefficients ranging typically from 0.75 to 0.85. Sodium retention is apparent on physical examination of oedema and ascites.

Study design

After stabilization of proteinuria, i.e. 6 weeks after induction of nephrosis, rats were stratified into two groups according to the mean of the proteinuria in weeks 5 and 6. Forty-five rats were treated with the ACE-inhibitor lisinopril (75 mg per litre of drinking water) and the remaining 15 were treated with vehicle. Treatment was continued for 2 weeks. The dose of lisinopril was based on previous studies in our laboratory demonstrating that this dose yields the maximum antiproteinuric effect [1].

Blood collection

Before and after the treatment period (weeks 6 and 8, respectively) blood was collected by orbital puncture under general anaesthesia with Isoflurane/O₂:N₂O. Blood samples were collected in heparin-containing vials and centrifuged immediately after collection at 4°C. Plasma obtained was stored at −20°C.

Measurements

Proteinuria was determined by a biuret method (Bioquant®; Merck, Darmstadt, Germany). Cholesterol was determined by an enzymatic in vitro test (Cholesterol CHOD-PAP®; test packet of Boehringer Mannheim GmbH, Mannheim, Germany).

Data analysis

Data are expressed as median and 95% confidence interval (CI) of the median. The CI was calculated according to the Binomial distribution with probability = 0.5 [8]. Pre-treatment values of proteinuria are represented as mean proteinuria in weeks 5 and 6. Data on proteinuria were normalized by log-transformation.

Statistical analysis was performed by student’s t-test (paired when appropriate). Correlations were determined by the Pearson method. Multivariate analysis was performed with either residual proteinuria (log-transformed) or post-treatment systolic blood pressure as the dependent variable, and pre-treatment proteinuria (log-transformed), cholesterol and systolic blood pressure as covariates. Statistical significance was assumed at the 5% level. Statistical analysis was performed by SPSS version 8.0.

Results

Six weeks after induction of nephrosis, the median proteinuria of all animals studied was 744 mg/day (range 609–860 mg/day). At that time, plasma cholesterol was markedly elevated up to 10.4 mmol/l (range 8.0–12.6 mmol/l), reflecting the state of systemic nephrosis. As expected, cholesterol correlated positively with proteinuria (r² = 0.77, P < 0.001). Proteinuria, cholesterol and blood pressure remained stable during the following 6 weeks in the vehicle group. Treatment with lisinopril resulted in a significant reduction of blood pressure, proteinuria and cholesterol (Table 1).

There was considerable interindividual difference in antiproteinuric efficacy of lisinopril (range −90% to +2%) and consequently a wide range of residual proteinuria (47–1119 mg/day). Both pre-treatment proteinuria (r² = 0.89, P < 0.001) and pre-treatment cholesterol (r² = 0.75, P < 0.001), but not pre-treatment blood pressure (r² = 0.00, P = 0.7), were correlated

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Table 1. Pre- and post-treatment values (with ranges shown in parentheses) for proteinuria, plasma cholesterol and systolic blood pressure

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Lisinopril</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proteinuria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment (mg/day)</td>
<td>741 (468–910)</td>
<td>758 (609–890)</td>
</tr>
<tr>
<td>Post-treatment (mg/day)</td>
<td>726 (432–846)</td>
<td>275 (175–496)(^b)(^c)</td>
</tr>
<tr>
<td>Change (%)</td>
<td>5 (–3 to 11)</td>
<td>–62 (–70 to –48)(^b)(^c)</td>
</tr>
<tr>
<td><strong>Cholesterol</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment (mmol/l)</td>
<td>8.9 (5.5–13.8)</td>
<td>10.6 (8.8–12.9)</td>
</tr>
<tr>
<td>Post-treatment (mmol/l)</td>
<td>11.2 (5.6–13.8)</td>
<td>4.5 (3.4–6.9)(^d)(^e)</td>
</tr>
<tr>
<td>Change (%)</td>
<td>8 (–29 to 29)</td>
<td>–47 (–57 to –36)(^b)</td>
</tr>
<tr>
<td><strong>Systolic blood pressure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment (mmHg)</td>
<td>140 (136–145)</td>
<td>144 (139–149)</td>
</tr>
<tr>
<td>Post-treatment (mmHg)</td>
<td>132 (105–152)</td>
<td>104 (89–118)(^d)(^d)</td>
</tr>
<tr>
<td>Change (%)</td>
<td>5 (–23 to 9)</td>
<td>–29 (–36 to –21)(^b)</td>
</tr>
</tbody>
</table>

\(^a^P<0.001\) vs pre-treatment; \(^b^P<0.001\) vs vehicle; \(^c^P<0.05\) vs vehicle; \(^d^P<0.01\) vs vehicle.

Table 2. Multivariate analysis

<table>
<thead>
<tr>
<th>Source</th>
<th>Mean square</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected model</td>
<td>3.573</td>
<td>207.095</td>
<td>0.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.448</td>
<td>25.977</td>
<td>0.000</td>
</tr>
<tr>
<td>Pre-treatment proteinuria [1]</td>
<td>1.298</td>
<td>75.248</td>
<td>0.000</td>
</tr>
<tr>
<td>Pre-treatment cholesterol</td>
<td>0.214</td>
<td>12.381</td>
<td>0.000</td>
</tr>
</tbody>
</table>

The dependent variable is residual proteinuria (log-transformed values for pre-treatment and residual proteinuria are used). \(r^2\) of the model is 0.92.

Discussion

In accordance with previous findings [1,9], in this study of non-hypertensive established nephrosis, ACE inhibition induced a marked improvement in proteinuria and concomitant systemic nephrosis. Interindividual differences in therapeutic efficacy were considerable. Residual proteinuria—but not blood pressure—was determined by the severity of pre-treatment proteinuria with a modest added effect of systemic nephrosis, as estimated from plasma cholesterol.

Systemic nephrosis is a complex state, including sodium retention, hypoalbuminaemia, coagulation abnormalities and dyslipidaemia. These abnormalities are also closely interrelated. Plasma cholesterol level, for instance, is related to excessive hepatic biosynthetic activity, which is (at least partly) stimulated by the reduced oncotic pressure that results from low plasma albumin [10]. In this exploratory analysis we used cholesterol as a single marker of this complex state, which does not allow us to identify which particular component(s) of the nephrotic state may be involved. Obviously, the next step would be to identify these in studies intervening in specific components of the nephrotic state.
Studies applying reduction of cholesterol before start of ACE inhibition would be of interest, as data from experimental nephrosis [11] as well as human hypertension [12,13] suggest that increased levels of plasma cholesterol reduce the therapeutic efficacy of ACE inhibition. Moreover, recent data showed that co-treatment of simvastatin and lisinopril augments the antiproteinuric and renoprotective efficacy of the ACE inhibitor in a model of passive Heymann nephritis [14]. In line with our results, the additive effect of the statin might, at least partly, be explained by improvement of the antiproteinuric response to the ACE inhibitor. However, statins have pleiotrophic effects [15] that could also have been involved.

The effect of sodium status on efficacy of ACE inhibition is well-known [1,4,16]. Thus, greater volume expansion in the more severely nephrotic animals may have been involved. If so, one would have expected an effect of nephrosis on blood pressure response also; however, neither proteinuria nor systemic nephrosis predicted the effects of ACE inhibition on blood pressure. This suggests that the observed effect of systemic nephrosis on the renal therapy response is not mediated by volume status in this experimental set-up, with all animals on a low sodium diet.

On the other hand, recent data suggest that the hyperlipidaemia itself could contribute to renal therapy resistance. Hyperlipidaemia is well known to be associated with increased renal damage in proteinuric models [17,18]. It was shown earlier that diet-induced hypercholesterolaemia induced early glomerular and interstitial changes and alterations in gene expression in non-proteinuric rats [19–22], which are similar to those observed in early phases of proteinuria-induced renal damage [23]. These precursors of renal damage may affect antiproteinuric efficacy of ACE inhibition. Also in human renal disease there is evidence that renal damage, present at the start of the treatment, can affect antiproteinuric efficacy of ACE inhibition [24].

Our data were obtained in adriamycin nephrosis, a rat model characterized by severe proteinuria and correspondingly severe metabolic derangements, as apparent from the cholesterol values that were four to six times the upper limit of normal. Within this context, pre-treatment proteinuria was a predictor of therapy response. In humans, a relationship between pre-treatment proteinuria and therapy response is not usually reported. One small study reported a relationship between a higher pre-treatment proteinuria and a worse antiproteinuric response [25], with a baseline proteinuria of 6 g/day in responders vs 13 g/day in non-responders. Our own group reported a similar percentage reduction in proteinuria in two small patient groups with a pre-treatment proteinuria of 7.4 g/day and 13.8 g/day, respectively, but with similar pre-treatment cholesterol [26]. In two reports from the Ramipril Efficacy in Nephropathy Trial study the percentage reduction in proteinuria from baseline was 13% in patients with baseline proteinuria 1–3 g/day [27], as compared with 23–55% in patients with baseline proteinuria ≥3 g/day [28], suggesting that baseline proteinuria and/or systemic nephrosis do not adversely affect the response to ACE inhibition in man. In patients with proteinuria ≥3 g/day, however, the metabolic derangements were relatively modest, as apparent from a mean baseline cholesterol of 6.69 mmol/l. Finally, in most human studies, there is considerable heterogeneity in diagnosis, duration of disease and renal damage at onset of treatment. All these factors could interfere with therapy response [24,29,30], as could differences in sodium status and protein intake [4,31]. Thus, from the data in humans, it is difficult to establish whether proteinuria as such or its systemic sequelae can be involved in responsiveness to therapy. For this reason the present study was done in a rat model, which allows standardization of type of renal damage and duration of disease. Whether our findings also apply to less severe nephrosis remains to be established.

The primary end point of this study was residual proteinuria after 2 weeks of antiproteinuric treatment. This time-point was chosen as in our model of established adriamycin nephrosis, the antiproteinuric response to ACE inhibition is stabilized and residual proteinuria predicts long-term renoprotection against renal structural damage [1], which is in accordance with other studies in experimental [32] and human [29,33] renal disease. Thus, our data may bear an impact on long-term renoprotection as well, but further long-term studies are required to support this assumption.

In conclusion, in this model of proteinuria-induced renal damage, not only the severity of proteinuria, but also the severity of systemic nephrosis provides a contribution, albeit modest, to renal therapy resistance, estimated as residual proteinuria, during ACE inhibition. Further studies, applying specific interventions in the proteinuria-induced metabolic derangements, are needed to identify the specific components of the metabolic derangements that could be causally involved in the modulation of the ACE-inhibition response. This may help to design treatment strategies aimed at improving therapeutic benefit in patients with proteinuric renal disease [34].

References
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