ACE inhibition restores glomerular charge selectivity to albumin in adriamycin nephrotic rats

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Introduction

Proteinuria has become an important determinant of progressive renal disease. Excessive filtration of proteins induces toxic effects to glomerular, mesangial, and tubular cells which results in glomerulosclerosis and tubulointerstitial fibrosis [1-3]. Clinical studies observed a relation between the degree of proteinuria and progressive loss of glomerular filtration rate in chronic renal disease [4-5]. Since reduction of proteinuria retards progressive loss of renal function in renal disease, it has become a therapeutically target [6]. A meta-analysis on comparative trials revealed that ACE inhibitors are superior to other antihypertensive agents with respect to their antiproteinuric capacity [7], whereas this greater antiproteinuric capacity of ACE inhibitors is recently associated with a better preservation of renal function in non-diabetic renal disease [8-9].

The mechanism of the antiproteinuric effect of ACE inhibition is not completely elucidated. Proteinuria is the consequence of a defect in the permeability of the glomerular capillary wall either glomerular size or charge selectivity being compromised [10]. Previous experimental and clinical studies showed improvement of glomerular size selectivity during the maximal antiproteinuric effect of ACE inhibition in non-diabetic renal disease [11-14]. It could be theoretically calculated that ACE inhibition improved glomerular size selectivity by intrinsic changes of the glomerular filtration barrier which are independent of hemodynamic alterations induced by ACE inhibition. This latter observation can be of importance for the mechanism of the typical time course of the antiproteinuric response during ACE inhibition. Acute administration of ACE inhibition reduces proteinuria by 10 to 20%, whereas continued treatment gradually reduced proteinuria further to 50 till 60% after approximately 4 weeks [15]. It has been suggested that the acute antiproteinuric response of ACE inhibition is mediated by blood pressure reduction [16]. A gradual restoration of glomerular permselectivity may mediate the additional increase of the antiproteinuric response during prolonged ACE inhibition, since it cannot be explained by changes in blood pressure or filtration fraction [15,17].

The antiproteinuric effect of ACE inhibition can be the result of restored glomerular charge selectivity, since we demonstrated that ACE inhibition increased the heparan sulfate content of the glomerular basement membrane in established adriamycin nephrosis [18]. However, no in vivo data on glomerular charge selectivity during the antiproteinuric response of ACE inhibition are available. The present study is set up to clarify whether effective reduction of proteinuria by ACE inhibition in adriamycin nephrotic rats is mediated by restoration of glomerular charge selectivity to albumin. In addition, it will be investigated whether this restoration of glomerular charge selectivity to albumin is a time-dependent phenomenon which explains the time course of the antiproteinuric response of ACE inhibition.

Methods
**Experimental design**

The study was performed in male Wistar rats of 250-300 g (Harlan, Zeist, The Netherlands). Adriamycin nephrosis was induced in 10 rats by intravenous injection of 2 mg/kg bodyweight adriamycin (Doxorubicin®, Pharmachemie BV, Haarlem, The Netherlands) at week 0. Since nephrotic-range proteinuria established after approximately 6 weeks [19], rats receive 5 mg/kg/day lisinopril in tap water from week 6 to 9. Subsequently, lisinopril was withdrawn from week 9 to 12. During the study all rats were housed in a temperature controlled room with a 12 hour light/dark cycle and were fed with a low sodium diet consisting of commercially available rat chow which contains 0.05% sodium chloride and 20% protein (Hope Farms Inc., Woerden, The Netherlands). They received fresh tap water daily ad libitum. During the study 24-hour urine protein excretion and systolic blood pressure were measured once weekly.

A time-controlled study was performed in 10 healthy rats which received a vehicle of 0.9% sodium chloride intravenously at week 0 as well as in 10 adriamycin nephrotic rats which were not treated with lisinopril.

**Renal clearance studies**

Renal clearance studies were performed in unrestrained and conscious rats in individual metabolic cages. We aimed to perform serial renal clearance studies in at least 6 rats of each group. All rats were instrumented one week before the first clearance study which allows one week recovery from surgery. After induction of anesthesia with 4% isoflurane and N₂:O₂ (1:2), the right jugular vein is catheterized with a silicone tube and a silicone catheter was implanted in the peritoneal cavity through the abdominal wall. The proximal ends of both catheters were tunneled subcutaneously and exteriorized on the head. The catheters were fixed on the skull by a modified method previously described by Steffens [20]. The catheters were filled with a 60% polyvinylpyrrolidone solution in saline with 500 IE/ml heparin closed with a piece of heat-sealed polyethylene tubing. Because of a high occlusion rate of jugular or intraperitoneal catheters in nephrotic rats, remaining rats of each group replaced rats with catheter occlusion during the further renal clearance studies. Adriamycin rats treated with lisinopril underwent renal clearance studies at baseline (week 6; n=6), and after 3 days (week 6½; n=6), 1 week (week 7; n=6), and 3 weeks of treatment with lisinopril (week 9; n=7), as well as after 3 days (week 9½; n=6), 1 week (week 10; n=6), and 3 weeks of withdrawal of lisinopril (week 12; n=5). Healthy control rats and untreated adriamycin rats underwent renal clearance studies at baseline (week 6) and at the end of the study (week 12).

Each renal clearance study started with a continuous infusion of 2 ml/hr dextrose 5% via the jugular vein from 7.00 a.m. to induce diuresis. At 9.00 a.m. each rat received a 0.3 ml bolus of 0.18 MBq ¹³¹I-native anionic rat serum albumin (RSA) and 0.18 MBq ¹²⁵I- charge modified neutral rat serum albumin (nRSA) via the jugular vein, whereas a 0.3 ml bolus of 0.22 MBq ⁹⁹Tc-DTPA was given via the
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intraperitoneal catheter followed by a 1 ml/hr continuous infusion of 0.8 MBq/hr $^{99m}$Tc-DTPA in dextrose 5% to estimate GFR. After an equilibration period of at least 2 hours, three clearance measurements were performed based upon spontaneous urine voiding. Blood samples (0.25 ml) were drawn via the jugular catheter to bracket each urine voiding.

Experimental and laboratory procedures

Twenty-four hour urine was collected in metabolic cages with free access to food and water. Urinary protein was determined by the biuret method [21]. Systolic blood pressure was measured in conscious rats by the tail-cuff method with an automated multichannel system (Apollo 179; IITC Life Science, Woodland Hills, Ca, USA) [22]. All animals were trained during two weeks before start of the study to become accustomed to the blood pressure measurements. Systolic blood pressure was taken as the mean of the last three of five measurements for each rat. Rats which participated in a renal clearance study did not undergo measurement of systolic blood pressure in a restrainer to prevent damage to the catheters fixed at their skull.

The preparation of charge-modified neutral rat serum albumin (nRSA) was carried out according to the method of Hoare and Koshland [23]. Carboxyl groups of native anionic rat serum albumin (RSA; molecular weight 68 kD; pl 4.8-5.1) are activated by water soluble 1-ethyl-3-(3-dimethyl-amino-propyl)-carbodiimide hydrochloride (EDC; Pierce, Rockford, Illinois, USA). The activated carboxyl groups react with the nucleophile ethylendiamine (EDA; Fisher Scientific Company, Far Lawn, New Jersey, USA). We prepared one batch of nRSA for the entire study. Fifty mg rat RSA (Sigma Chemical Co., St. Louis, MO, USA) was added to 1.67 ml EDA (0.9 g/l) and 11 ml distilled water. The solution was cooled at 25°C and titrated to a pH of 4.75 with 10 N hydrochloric acid. EDC (10 mg/ml) was slowly added during 15-30 minutes under stable conditions with respect to pH and temperature. The reaction continued for 120 minutes and then was ended by addition of 0.37 ml 4 M acetate buffer with pH 4.75. The solution was dialyzed at 4°C against distilled water during two days and finally lyophilized and stored at -80°C. The recovery of nRSA was 40.6 mg (80%).

RSA and nRSA were characterized with respect to their isoelectric point (pl) by isoelectrofocussing at commercially prepared PhastGel IEF media with a pl range from 3 to 9 (Pharmacia, Uppsala, Sweden) according to the manufacturer’s instructions. This charge modification procedure increased the pl of RSA from 5.0 (range: 4.6-5.1) to approximately 7.0 (range: 6.0-8.5) as shown in figure 1. The day before each clearance study 10 $\mu$l RSA and nRSA (100 mg/ml) were radiolabelled with $^{131}$I and $^{125}$I, respectively, by the chloramine-T method followed by a size-exclusion procedure at a Sephadex G25 column to separate radiolabelled albumin from free radioactive iodine [24]. Fractions containing radiolabelled RSA and nRSA were pooled and stored at 4°C. Autoradiography of PhastGel IEF gels did not reveal any change of molecular charge due to the radiolabelling of RSA or nRSA (Figure 1).
Figure 1. Molecular charge of rat serum albumin (RSA) and neutralized rat serum albumin (nRSA). The left figure shows RSA (c and f) and nRSA (b, e and h) before radiolabelling with iodine, whereas the right figure shows RSA (a and c) and nRSA (b and d) after radiolabelling with iodine.

Molecular size of RSA and nRSA was estimated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (Boehringer Ingelheim Bioproducts, Heidelberg, Germany) [25]. Figure 2 shows that charge modification of RSA did not alter its molecular size.

DTPA was radiolabelled with $^{99}$Tc just before the start of each clearance study. Blood samples obtained during the clearance study were collected in heparinized tubes and immediately centrifuged during 1 minute (16000 rpm). Distilled water was added to 100 µl plasma aliquots to a final volume of 580 µl and stored at 4°C. Urine samples obtained during the clearance study were collected in preweighed tubes and volumes were measured gravimetrically. Of each urine sample 100 µl was twice precipitated with 200 µl trichloroacetic acid (30%) after addition of 100 µl unlabelled RSA (2%) and distilled water to ensure a final volume of 580 µl. The final urine precipitate was solved in 200 µl 10 N NaOH and 380 µl distilled water. Addition of unlabelled RSA provides an optimal recovery of labeled RSA (>97%) after precipitation in urine samples with low protein concentration obtained during forced diuresis. The final sample volume has to be greater than 500 µl for optimal determination of radioactivity during 4 minutes in a two-channel scintillation counter (LKBG Compugamma Scintillation Counter, Wallace, Finland). Immediately after ending the clearance study, radioactivity of $^{99}$Tc-DTPA and $^{131}$I-
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RSA was determined in plasma and urine samples. Two days after counting, radioactivity of $^{131}$I-RSA and $^{125}$I-nRSA was determined in plasma and urine samples, since $^{99}$Tc-DTPA is completely decayed at this time. The glomerular filtration rate (GFR) is expressed as the renal DTPA clearance by the formula:

$$\frac{(U_{[\text{DTPA}]} \times V)}{P_{[\text{DTPA}]}},$$

where $U$ is counts/min per ml urine precipitate, $V$ is the urine volume in ml/min, and $P$ is counts/min per ml plasma. Fractional clearances of RSA and nRSA were calculated according to the formula:

$$\frac{U_{[\text{albumin}]} \times P_{[\text{DTPA}]}}{U_{[\text{DTPA}]} \times P_{[\text{albumin}]}}$$

Glomerular charge selectivity is represented by the renal clearance index of nRSA relative to RSA.

![Molecular size of rat serum albumin](image)

**Figure 2.** Molecular size of rat serum albumin (RSA; a) and neutralized rat serum albumin (nRSA; b).

**Data analysis**

Data are expressed as mean and standard deviation (SD), unless otherwise indicated. Differences in proteinuria, systolic blood pressure, GFR, nRSA and RSA clearances as well as their index during treatment and withdrawal of lisinopril were
tested with the Kruskal-Wallis nonparametric ANOVA test followed by Duncan’s correction for multiple comparisons. Differences between RSA and nRSA clearances at a given time-point were tested with the Wilcoxon rank sum test for paired observations. Differences between adriamycin rats treated with lisinopril and untreated adriamycin rats or control rats at week 6 and 12 were tested by the Mann-Whitney test for unpaired observations. Statistical significance was assumed at a p-level less than 0.05.

Results

Time-control studies revealed no differences in systolic blood pressure or GFR at baseline or at the end of the study between healthy rats, adriamycin rats to be untreated, and adriamycin rats to be treated with lisinopril (Table 1). Both groups of adriamycin rats demonstrated significantly higher proteinuria as well as fractional nRSA and RSA clearances in comparison to healthy rats. The adriamycin rats to be treated with lisinopril showed a stable proteinuria six weeks after adriamycin injection (Figure 3). The increase of fractional RSA clearance is 3 to 6 times higher than the increase of nRSA clearances in adriamycin rats. Comparison of these parameters in both groups of adriamycin rats revealed no differences, except for a higher fractional nRSA clearance at baseline in adriamycin rats to be treated with lisinopril. Both group of adriamycin rats have an impaired glomerular charge selectivity as reflected by a reduced nRSA to RSA clearance index in comparison to healthy rats (0.4 and 0.7 versus 2.4, respectively).

ACE inhibition

Lisinopril reduced systolic blood pressure (123±10 to 83±10 mmHg; p<0.05), whereas GFR did not significantly change (1.6±0.4 to 0.9±0.5 ml/min). Blood pressure was maximally reduced after 1 week treatment and did not fall further. As depicted in figure 3, proteinuria gradually fell from 276±129 to 83±106 mg/24hr (p<0.05). Both the fractional RSA and nRSA clearances significantly fell in such a way that they did not differ significantly from each other anymore. Since the decrease of fractional RSA clearance was 2.4 times greater than that of nRSA, the nRSA to RSA clearance index rose from 0.7±0.7 to 1.7±1.5 (p<0.01). Fractional nRSA and RSA clearances as well as the nRSA to RSA clearance index in adriamycin rats after 3 weeks lisinopril were comparable to those in healthy rats. The change in proteinuria was significantly related with the concomitant change in the nRSA to RSA clearance index during lisinopril (Figure 4).
Figure 3. Time course of primary study parameters in adriamycin rats treated with lisinopril. The upper part shows proteinuria in all 10 rats during the entire study (closed circles) as well as proteinuria in adriamycin rats during the renal clearance experiments (bars). The middle part shows fractional nRSA (closed circles) and RSA (open squares) clearances. The lower part shows the nRSA to RSA clearance index (bars). ACEi = ACE inhibition by lisinopril. *p<0.05 versus wk 6, #p<0.05 versus wk 9, **p<0.05 nRSA versus RSA
Table 1. Experimental data of healthy rats (CON), untreated adriamycin rats (ADR), and adriamycin rats treated with lisinopril (ADR+ACEi) at baseline (wk 6) and at the end of the study (wk 12).

<table>
<thead>
<tr>
<th></th>
<th>CON (wk6)</th>
<th>CON (wk12)</th>
<th>ADR (wk6)</th>
<th>ADR (wk12)</th>
<th>ADR+ACEi (wk6)</th>
<th>ADR+ACEi (wk12)</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>U&lt;sub&gt;protein&lt;/sub&gt; (mg/day)</td>
<td>15±10</td>
<td>18±9</td>
<td>263±55*</td>
<td>381±96*</td>
<td>300±138*</td>
<td>471±311*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>114±10</td>
<td>129±9</td>
<td>121±13</td>
<td>126±11</td>
<td>123±10</td>
<td>119±4</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>1.4±0.3</td>
<td>1.1±0.4</td>
<td>1.2±0.1</td>
<td>0.8±0.4</td>
<td>1.6±0.4</td>
<td>0.6±0.2*</td>
</tr>
<tr>
<td>Cl&lt;sub&gt;nRSA&lt;/sub&gt; (10&lt;sup&gt;-5&lt;/sup&gt;)</td>
<td>11±3</td>
<td>27±2*</td>
<td>37±26*§</td>
<td>136±39*§</td>
<td>206±126*§</td>
<td>465±520*§</td>
</tr>
<tr>
<td>Cl&lt;sub&gt;RSA&lt;/sub&gt; (10&lt;sup&gt;-5&lt;/sup&gt;)</td>
<td>5±1</td>
<td>12±3*</td>
<td>110±53*</td>
<td>498±178*#</td>
<td>522±343*</td>
<td>1480±1625*</td>
</tr>
<tr>
<td>nRSA/RSA</td>
<td>2.4±0.8</td>
<td>2.5±1.0</td>
<td>0.4±0.1*</td>
<td>0.3±0.1*</td>
<td>0.7±0.7*</td>
<td>0.4±0.1*</td>
</tr>
</tbody>
</table>

Abbreviations are: U<sub>protein</sub> = proteinuria; SBP = systolic blood pressure; GFR = glomerular filtration rate; Cl<sub>nRSA</sub> = fractional clearance of 125<sup>I</sup>-charge-modified rat albumin; Cl<sub>RSA</sub> = fractional clearance of 131<sup>I</sup>-native rat albumin. *p<0.05 versus CON at corresponding time; #p<0.05 wk12 versus wk6 within each group; §p<0.05 ADR versus ADR+ACEi at corresponding time; $p<0.05$ Cl<sub>nRSA</sub> versus Cl<sub>RSA</sub> at corresponding time.

In addition, the change in proteinuria, fractional RSA and nRSA clearances, and the nRSA to RSA renal clearance index showed a similar time course during ACE inhibition (Figure 3).

**Recovery**
Withdrawal of lisinopril induced a complete recovery of systolic blood pressure (83±10 to 119±4 mmHg; p<0.05), whereas GFR had significantly decreased at week 12 (0.9±0.5 to 0.6±0.2 ml/min). Proteinuria also returned to baseline values after 3 weeks withdrawal of lisinopril. Although both the fractional RSA and nRSA clearance significantly increased after withdrawal of lisinopril, the fractional clearance of RSA is significantly higher than that of nRSA at the end of the study. The nRSA to RSA clearance index completely recovered from 1.7±1.5 to 0.4±0.1 (p<0.05), since the increase of fractional RSA clearance was 2.9 times greater than that of nRSA. The recovery of proteinuria, fractional RSA and nRSA clearances, and the nRSA to RSA clearance index again showed a similar time course which is a reflection of the time course during the antiproteinuric response of lisinopril.
Discussion

The antiproteinuric effect of ACE inhibition is superior to that of other antihypertensive drugs with equal capacity to reduce blood pressure [7]. This greater capacity to reduce proteinuria is associated with a better preservation of renal function in non-diabetic renal disease [8-9]. The mechanism of the antiproteinuric effect of ACE inhibition has not completely been elucidated so far. The present study demonstrates that effective reduction of proteinuria by the ACE inhibitor lisinopril is associated with a restoration of glomerular charge selectivity to albumin in the adriamycin nephrotic rat. This restoration of glomerular charge selectivity even demonstrates a similar time course as the reduction of proteinuria by lisinopril. These observations implicate that restoration of glomerular charge selectivity contributes to the favorable antiproteinuric effect of ACE inhibitors.

![Graph showing correlation between change in proteinuria and change in the nRSA to RSA clearance index during ACE inhibition.](image)

*Figure 4. Correlation between change in proteinuria and change in the nRSA to RSA clearance index during ACE inhibition (n=12; r=-0.70; p<0.05).*

Previous studies on the mechanism of the antiproteinuric effect of ACE inhibition in non-diabetic renal disease focused on the role of alterations in hemodynamics or glomerular size selectivity. The acute antiproteinuric response of ACE inhibition is induced by systemic blood pressure reduction through reduced generation of systemic angiotensin II [16]. Continued treatment with ACE inhibition
gradually increases the antiproteinuric response from 15 to 50% in 4 weeks time, whereas blood pressure or filtration fraction do not further fall [15]. In addition, reversal of blood pressure and filtration fraction by systemic infusion of angiotensin II does not reverse the established antiproteinuric response of ACE inhibition [17]. It therefore is unlikely that hemodynamic alterations directly mediate the additional reduction of proteinuria during continued ACE inhibition. In accordance, ACE inhibition restores glomerular size selectivity to dextran at its maximal antiproteinuric effect independently from its hemodynamic alterations [11-14]. Theoretical analysis of intrinsic permeability characteristics of the glomerular capillary wall during ACE inhibition reveals a reduction in mean pore size as well as a reduction of pore size distribution which restricts the passage of large macromolecules [12]. ACE inhibition may therefore induce structural changes of the glomerular filtration barrier through indirect effects of hemodynamic changes which reduce the intraglomerular capillary wall stress or by inhibition of intrarenal angiotensin II generation [26].

Proteinuria in non-diabetic renal disease is associated with impaired glomerular charge selectivity which may be due to a reduction of anionic glycosaminoglycan side chains of heparan sulfate proteoglycan in the lamina rarae of the glomerular basement membrane [27-30]. This reduction of heparan sulfate proteoglycan may increase glomerular permeability by the interference with the attachment of glomerular epithelial cells to the glomerular basement membrane which leads to retraction or detachment of the epithelial cells [31]. Although restoration of glomerular charge selectivity may contribute to the antiproteinuric response of ACE inhibition, no studies have been published so far which analyzed glomerular charge selectivity during any antiproteinuric treatment. This lack of data is probably the consequence of a limited sensitivity of the available methods to measure changes in glomerular charge selectivity. Although dextran sulfate is the only available marker of glomerular charge selectivity of which the fractional clearance is not affected by tubular reabsorption or secretion, its fractional clearance is affected by desulfatation during the filtration process, it is toxic in high concentration, it binds size-dependent to serum proteins, and its molecular size of 18Å does not match the molecular size of albumin [27, 32-33]. All other available markers of glomerular charge selectivity such as differently charged horseradish peroxidases [34], IgG subclasses [35], and amylase iso-enzymes [36] are proteins which are subject to charge-dependent tubular reabsorption [37]. In addition, anionic horseradish peroxidase is more prone to degradation during the filtration process than neutral or cationic horseradish peroxidase [38]. We decided to use fractional clearances of differently charged autologous albumin to monitor glomerular charge selectivity, since albumin is the naturally occurring plasma protein for charge dependent filtration. It is known that the charge modification procedure which increases pI of albumin does not alter its molecular size and does not induce degradation of albumin during filtration [39]. Of course, use of differently charged albumin’s for the monitoring of glomerular charge selectivity also inherits drawbacks. Infusion of highly cationized albumin induces renal and extrarenal toxicity [40]. The
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risk for this toxicity is negligible in the present study since molecular charge of albumin was only neutralized. More importantly, fractional albumin clearances are influenced by the charge-dependent tubular reabsorption of albumin [37]. Micropuncture studies in healthy rats demonstrated more than 90% tubular reabsorption of albumin, whereas cationic albumin with a pI greater than 9.0 is 5 times more reabsorbed than native anionic albumin during infusion of low albumin concentrations in the proximal tubule [37, 41]. Since we did not correct for this charge-dependent tubular handling of albumin, the nRSA to RSA clearance index has to be underestimated in our healthy rats. Tubular reabsorption of albumin decreases progressively below 20% in nephrotic conditions, whereas the reabsorption of highly cationic albumin is 2-3 times higher than that of anionic albumin when higher albumin concentrations are infused in proximal tubules [37, 42]. Bertolatus et al. [39] observed a smaller difference in tubular reabsorption between anionic BSA and neutral BSA (31 versus 42%, respectively) in adriamycin nephrotic rats. Since we modified molecular charge of RSA in the same order, nRSA may thus be preferentially reabsorbed which explains the higher fractional clearance of RSA compared to nRSA in our adriamycin rats at baseline. It is not likely that differences in molecular size or configuration due to the charge modification procedure of albumin induced higher fractional clearance of RSA than nRSA. Molecular size of nRSA is equal or even slightly smaller than that of RSA which excludes significant polymerization of charge-modified albumin molecules leading to smaller fractional clearances of nRSA. Cationization of albumin to a pI of 9.3 to 9.5 induced changes in molecular configuration due to a 50% loss of $\alpha$-helical content of the native molecule [43]. This would rather facilitate than attenuate the renal clearance of highly cationized RSA in comparison to RSA in adriamycin rats at baseline. Furthermore, the mild neutralization of albumin in the present study would not have similar configurational effects. The relatively higher clearance of RSA in comparison to nRSA which we observed in adriamycin rats at baseline has therefore to reflect impaired glomerular charge selectivity. It is known that effective reduction of proteinuria by ACE inhibition increases the tubular reabsorption of albumin due to a smaller tubular protein load [44]. Since lower albumin concentrations at tubular level facilitates the tubular reabsorption of nRSA more than RSA [37], the increase of the nRSA to RSA clearance index during lisinopril is even underestimated in the present study and therefore reflects a restoration of glomerular charge selectivity to albumin.

Would glomerular size selectivity restore with a similar time course as glomerular charge selectivity in the adriamycin rat? The nature of the permselective defect of the glomerular capillary wall in the adriamycin rat seems to depend on the stage of the induced nephrosis [45]. Adriamycin induces a rapid and sustained reduction of heparan sulfate charge density and epithelial membrane sialic acid content in the glomerular capillary wall which precedes onset of proteinuria [46]. This is followed by focal effacement of foot processes of podocytes and detachment of podocytes from the glomerular basement membrane which leads to established nephrotic-range proteinuria within 4 to 6 weeks. Impaired glomerular charge selectivity has a predominant role at this stage of adriamycin nephrosis [39, 45].
Glomerular size selectivity may be impaired in later stages of adriamycin nephrosis since progressive focal and segmental glomerulosclerosis just develops from 12 to 16 weeks after induction of nephrosis [47-48]. We were unable to simultaneously measure glomerular size and charge selectivity in the present study since this would too much complicate our renal clearance protocol and laboratory assays. Nevertheless, the simultaneous increase of fractional RSA and nRSA clearances in adriamycin rats at baseline suggests the concomitant presence of impaired glomerular size selectivity, whereas the simultaneous reduction of fractional RSA and nRSA clearances during lisinopril suggests a concomitant restoration of glomerular size selectivity. A time-dependent restoration of glomerular size selectivity may therefore also contribute to the effective reduction of proteinuria by ACE inhibition in adriamycin nephrotic rats.

In conclusion, restoration of glomerular charge selectivity to albumin is an important determinant of the antiproteinuric response of ACE inhibition in adriamycin nephrosis. The time course of the changes in glomerular charge selectivity and proteinuria during ACE inhibition suggest a gradual restoration of structural anionic sites in the glomerular capillary wall. Other interventions which restore structural anionic sites of the glomerular capillary wall may extend the reduction of proteinuria in renal disorders which are predominantly characterized by impaired glomerular charge selectivity.

Acknowledgments

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