Renal accumulation of pentosidine in non-diabetic proteinuria-induced renal damage in rats

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Abstract

Background. Advanced glycation end-products (AGEs) contribute to the pathogenesis of diabetic glomerulopathy. The role of AGEs in non-diabetic renal damage is not well characterized. First, we studied whether renal AGE accumulation occurs in non-diabetic proteinuria-induced renal damage and whether this is ameliorated by renoprotective treatment. Secondly, we investigated whether renal AGE accumulation was due to intrarenal effects of local protein trafficking.

Methods. Pentosidine was measured (by high-performance liquid chromatography) in rats with chronic bilateral adriamycin nephropathy (AN), untreated and treated with lisinopril. Age-matched healthy rats served as negative controls. Secondly, we compared renal pentosidine in mild proteinuric and non-proteinuric kidneys of unilateral AN and in age-matched controls at 12 and 30 weeks. Intrarenal localization of pentosidine was studied by immunohistochemistry.

Results. Renal pentosidine was elevated in untreated AN (0.14 ± 0.04 μmol/mol valine) vs healthy controls (0.04 ± 0.01 μmol/mol valine, P < 0.01). In lisinopril-treated AN, pentosidine was lower (0.09 ± 0.02 μmol/mol valine) than in untreated AN (P < 0.05). In unilateral proteinuria, pentosidine was similar in non-proteinuric and proteinuric kidneys. After 30 weeks of unilateral proteinuria, pentosidine was increased in both kidneys (0.26 ± 0.10 μmol/mol valine) compared with controls (0.18 ± 0.06 μmol/mol valine, P < 0.05). Pentosidine (AN, week 30) was also increased compared with AN at week 12 (0.16 ± 0.06 μmol/mol valine, P < 0.01). In control and diseased kidneys, pentosidine was present in the collecting ducts. In proteinuric kidneys, in addition, pentosidine was present in the brush border and cytoplasm of dilated tubular structures, i.e. at sites of proteinuria-induced tubular damage.

Conclusion. Pentosidine accumulates in non-diabetic proteinuric kidneys in damaged tubules, and renoprotective treatment by angiotensin-converting enzyme (ACE) inhibitors inhibits AGE accumulation, supporting a relationship between abnormal renal protein trafficking, proteinuria-induced tubular damage and tubular pentosidine accumulation. Future studies, applying specific AGE inhibitors, should be conducted to provide insight into the pathophysiological significance of renal AGEs in non-diabetic renal disease.

Keywords: Advanced glycation end products; Maillard reaction; Nonenzymatic glycosylation; adriamycin nephrosis; angiotensin converting enzyme (ACE) inhibition; immunohistochemistry

Introduction

Several lines of evidence support a pathophysiological role for advanced glycation end-products (AGEs) in diabetic nephropathy. First, accumulation of AGEs occurs in glomerular and tubulointerstitial compartments in experimental diabetic nephropathy in proportion to the severity of renal damage [1], and formation of AGEs precedes and correlates with early manifestations of renal disease in diabetes [2]. Moreover, AGEs are nephrotoxic in vitro and in vivo [3,4]. Finally, pharmacological intervention in AGE formation
protects against structural lesions, proteinuria and renal function loss in experimental diabetes [5].

The role of AGEs in non-diabetic renal damage, however, is less well characterized. Yet, renal accumulation of AGEs could play a role in non-diabetic renal damage as well. In uremic patients, AGE levels are increased in plasma and tissue proteins, independent of hyperglycaemia [6]. This might be related to increased AGE formation as a result of oxidative stress [6], or dyslipidaemia [7] and/or decreased renal clearance of AGEs [8]. Moreover, inhibition of AGE formation protects against age-related renal function loss in normal rats [9] and has been shown to protect against renal pathology in non-diabetic Zucker obese rats [7].

Therefore, in the present study, we investigated first whether pentosidine, a well characterized AGE, accumulates in the kidney in adriamycin-induced proteinuria and, secondly, whether this possible renal accumulation is attenuated by antiproteinuric treatment. Moreover, to investigate whether abnormal intrarenal protein trafficking, which is characteristic of the proteinuric state, might be involved in the alleged renal AGE accumulation, we studied renal pentosidine in unilateral adriamycin-induced proteinuria and compared the proteinuric with the non-proteinuric kidney. Finally, immunohistochemistry was used to evaluate the intrarenal localization of pentosidine. The AGE pentosidine was selected for measurement because it is a known independent variable for the presence of ischaemic heart diseases and hypertension [10]. Moreover, increases in plasma pentosidine are associated with arteriosclerosis [11], which shares many similarities with focal and segmental glomerulosclerosis, suggesting relevance to renal pathology.

Materials and methods

Experimental groups

Adult male Wistar rats were studied (Hsd.Cpb. Wu; Harlan Inc., Zeist, The Netherlands). The animals were housed in a temperature-controlled room, with a 12 h light/dark cycle and had free access to food and water. The Committee for Animal Experiments of the University Medical Center and had free access to food and water. The Committee for Animal Experiments of the University Medical Center approved the experiments.

Experiment I: bilateral adriamycin-induced proteinuria \( (n = 12) \)

Bilateral proteinuria group + ACE inhibitor \( (n = 4) \). According to our standard regimen, all rats received a low sodium diet (0.05% NaCl, 20% protein, Hope Farms Inc., Woerden, The Netherlands) to obtain optimal therapeutic efficacy of angiotensin-converting enzyme (ACE) inhibition [12]. Nephrotic syndrome was induced by injection of 2 mg/kg adriamycin in the tail vein under light isoflurane anaesthesia. Treatment with lisinopril (75 mg/l drinking water) started 6 weeks afterwards, i.e. after stabilization of proteinuria, until the end of the study. Rats were sacrificed in week 15.

Bilateral proteinuria group untreated \( (n = 4) \). All rats underwent the same procedure as the previous group, but with vehicle treatment. Rats were sacrificed in week 15.

Healthy controls \( (n = 4) \). Age-matched healthy control rats were used to study the effect of ageing on AGE accumulation. Rats were sacrificed in week 15.

Experiment II: unilateral adriamycin-induced proteinuria \( (n = 18) \)

Unilateral proteinuria group, adriamycin nephropathy \( (n = 12) \). Unilateral proteinuria was induced by exposing only one kidney to adriamycin toxicity by briefly clamping the contralateral renal artery during adriamycin injection, as described previously [13]. In this model, renal damage is absent in the clipped kidney. In the adriamycin-exposed kidney, proteinuria develops, subsequently resulting in structural damage, with, however, a milder development than in bilateral proteinuria for any given severity of proteinuria per kidney [13]. Therefore, in this experiment, follow-up was extended to 30 weeks, with sacrifice of half of the rats at week 12, to evaluate the time course of changes.

Unilateral proteinuria group, saline control \( (n = 6) \). These rats also underwent surgery, but were injected with saline and served as negative time controls. Half of the rats were sacrificed at week 12, the other half at week 30, to evaluate the time course of changes.

Blood pressure, proteinuria, creatinine and cholesterol measurements

Body weight, systolic blood pressure (SBP) and proteinuria were measured weekly. SBP was measured weekly after 2 weeks of daily training prior to induction of nephrosis. An automated multichannel system was used with tail cuffs and photoelectric sensors to detect the tail pulse (Apollo 179; IITC Life Science. Woodland Hills, CA). The rats were placed in a test chamber in restrainers while the temperature was maintained at 27–29°C. For each rat, the value was calculated from the mean of two consecutive measurements.

The urinary protein was determined weekly with the Biuret method, from 24h urine collected in metabolic cages (BioquantTM; Merck, Darmstadt, Germany). Creatinine and cholesterol concentrations in plasma were determined from blood collected at the end of the experiment, prior to sacrifice. Analyses were performed by an automatic analyser (Synchron CX7, Beckman Coulter Inc., Fullerton, CA).

Tissue processing and renal morphology

At the end of the studies, kidneys were perfused with saline, the animals were sacrificed, and the kidneys were harvested for histological examination. Tissue was fixed in 4% paraformaldehyde and processed for paraffin embedding. Sections (4 μm) were stained with periodic acid–Schiff and examined by light microscopy in a blinded fashion to evaluate focal glomerulosclerosis (FGS). The degree of FGS was assessed in 50 glomeruli by semi-quantitative scoring on a scale of 0–4. FGS was scored positive when mesangial matrix expansion and adhesion of the glomerular visceral epithelium to Bowman’s capsule were present in the same quadrant.
If 25% of the glomerulus was affected, a score of 1 was given, 50% was scored as 2, 75% as 3, and 100% as 4. The ultimate score is then obtained by multiplying the degree of change by the percentage of glomeruli with the same degree of injury and addition of these scores, thus rendering a theoretical range of 0–200.

**Immunohistochemistry**

Formalin-fixed, paraffin-embedded tissues were cut at 4 μm on 3-aminopropyltriethoxysilane (Sigma)-coated slides, deparaffinized, and rehydrated in a graded series of ethanol. Sections were soaked in 100% methanol containing 0.3% H2O2 for 10 min to block endogenous peroxidase. After that, sections were incubated with 0.05% pronase (DakoCytomation, Denmark, catalogue no. S 2013) in TBS (Tris borate buffer) for 15 min at 37°C. A peroxidase conjugated-secondary antibody, followed by a 3,3'-diaminobenzidine solution containing 0.003% H2O2 for 10 min to develop the peroxidase activity. Sections were counterstained with haematoxylin and mounted. Control sections, incubated with anti-pentosidine antibody solutions, after being pre-incubated with the antigen to which it is directed, i.e. pentosidine–bovine serum albumin (BSA) were negative.

**Pentosidine measurements by high-performance liquid chromatography (HPLC)**

**Preparation of sample.** Kidney cortex tissue (200 mg) was minced and homogenized in 4 ml of phosphate-buffered saline containing 5 mM diethylenetriaminepenta-acetic acid at 4°C. Protein, 7.5 mg based on the Lowry assay, was reduced with a final concentration of 400 mM sodium borohydride to prevent further AGE formation during acid hydrolysis. After precipitation of protein with an equal volume of 20% trichloroacetic acid, the protein was delipidated by extraction with methanol:ether [(v:v) 3:1]. Samples were then dialysed overnight at 4°C against deionized water. After drying the samples using a Speed-Vac centrifugal evaporator system (Savant Instruments, Farmingdale, NY), 4 ml of 6 N HCl was added and the samples were hydrolysed in 6 N HCl at 110°C for 20 h. The hydrolysates were dried using the Speed-Vac and then dissolved in 500 μl of H2O. 20 μl was removed for amino acid analysis. To clean up the samples and recover pentosidine, the samples were applied to a 1 ml SP-Sephadex C-25 column (Amersham Pharmacia, Biotech AB, Sweden), and the pentosidine eluted in 5 ml of 1 N HCl, followed by overnight at 4°C against deionized water. After drying the samples using a Speed-Vac centrifugal evaporator system (Savant Instruments, Farmingdale, NY), 4 ml of 6 N HCl was added and the samples were hydrolysed in 6 N HCl at 110°C for 20 h. The hydrolysates were dried using the Speed-Vac, and then dissolved in 500 μl of H2O; 20 μl was removed for amino acid analysis. To clean up the samples and recover pentosidine, the samples were applied to a 1 ml SP-Sephadex C-25 column (Amersham Pharmacia, Biotech AB, Sweden), and the pentosidine eluted in 5 ml of 1 N HCl, followed by drying as above. The samples were then dissolved in 200 μl of 1% heptafluorobutyric acid (HFBA) for HPLC analysis.

**HPLC analysis.** Pentosidine content of renal cortical homogenates was measured as described previously [15]. A 60 μl aliquot was injected into the HPLC system and separated on a C-18 reverse phase column (25 × 0.46 cm, Keystone 255-33, 120 Å pore size). Water–HFBA–acetonitrile was used as mobile phase. The effluent was monitored with a fluorescence detector (RFA-10A; Shimadzu, Kyoto, Japan) at excitation-emission wavelengths of 328/378 nm. The amount of pentosidine present in the sample was calculated based on the area units for the synthetic pentosidine standard and was normalized to the valine content of the hydrolysate measured by amino acid analysis, using cation exchange chromatography.

**Data analysis**

Data are expressed as the mean ± SD. For experiment I, statistical analysis of group differences was performed by a Kruskal–Wallis analysis of variance on ranks. For experiment II, differences between the proteinuric and non-proteinuric kidney in unilateral proteinuria, between the saline group and the adriamycin group and between week 12 and week 30 were tested using a Student t-test. To analyse for the association between pentosidine accumulation and renal damage, data from the different groups from experiment I were pooled and analysed non-parametrically using Spearman’s rho correlation coefficient. Statistical significance was assumed at the 5% level.

**Results**

**Body weight**

Throughout the experiments, food and water intake was similar in all groups, consistent with a good condition of the nephrotic animals. No differences in body weight were observed between the groups in either experiment (Tables 1 and 2).

**Blood pressure**

**Bilateral proteinuria.** SBP remained stable after induction of nephrosis until the start of treatment. Lisinopril induced a significant drop in blood pressure from 150 ± 6 to 97 ± 2 mmHg at the end of the study (P < 0.001), whereas the untreated nephrotic group (158 ± 6 to 148 ± 9 mmHg) and the healthy control group (120 ± 8 to 108 ± 16 mmHg) showed no change in blood pressure throughout the study (Table 1).

**Table 1. Results of experiment I: bilateral adriamycin-induced proteinuria**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 4)</th>
<th>AN (n = 4)</th>
<th>AN + ACEi (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>440 ± 30</td>
<td>445 ± 22</td>
<td>427 ± 25</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>108 ± 16</td>
<td>148 ± 9</td>
<td>97 ± 2</td>
</tr>
<tr>
<td>Proteinuria (mg/day)</td>
<td>15 ± 7</td>
<td>722 ± 61a</td>
<td>52 ± 12a</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>73 ± 4</td>
<td>67 ± 8</td>
<td>60 ± 21</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>1.52 ± 0.46</td>
<td>9.74 ± 1.12a</td>
<td>3.00 ± 0.30a</td>
</tr>
<tr>
<td>Pentosidine/valine (μmol/mol)</td>
<td>0.04 ± 0.01</td>
<td>0.14 ± 0.04a</td>
<td>0.09 ± 0.02a</td>
</tr>
<tr>
<td>Focal glomerulosclerosis</td>
<td>0 ± 0</td>
<td>50 ± 30</td>
<td>3 ± 5</td>
</tr>
</tbody>
</table>

Parameters at the end of the study are shown. All values are expressed as mean ± SD. AN = adriamycin nephrosis; ACEi = angiotensin-converting enzyme inhibition. Focal glomerulosclerosis is expressed in arbitrary units. *P < 0.05 vs control. **P < 0.05 vs AN + ACEi.
Unilateral proteinuria. From the start of the experiment until week 30, we observed a significant rise in blood pressure in both the unilateral proteinuria (125±11 to 159±20 mmHg, \( P < 0.01 \)) and the saline group (133±21 to 164±21 mmHg, \( P < 0.05 \)), without differences between these untreated groups (Table 2). Thus, unilateral proteinuria did not affect the progression of hypertension in these rats.

Urinary protein excretion and creatinine

Proteinuria developed rapidly during the 4 weeks after adriamycin injection and subsequently levelled off and stabilized. As anticipated, in rats with untreated bilateral disease (Table 1), proteinuria was approximately twice as high as in rats with unilateral proteinuria (Table 2).

Bilateral proteinuria. Lisinopril induced a significant reduction in proteinuria, from 530±105 mg/day at week 6 (start of treatment) to 52±12 mg/day at the end of the study (\( P < 0.01 \)), whereas untreated rats showed a modest further rise in proteinuria towards the end of the study, from 555±172 to 722±61 mg/day. Proteinuria in the lisinopril group remained slightly, but significantly higher than in healthy control rats (15±7 mg/day, \( P < 0.05 \)) at the end of the study (Table 1).

Unilateral proteinuria. In the unilateral proteinuria group, proteinuria was significantly elevated vs saline controls at both week 12 (261±105 vs 26±14, \( P < 0.05 \)) and week 30 (326±107 vs 33±26, \( P < 0.01 \)) (Table 2).

No significant differences in serum creatinine were observed between the groups in either experiment (Tables 1 and 2).

Renal structural changes

Bilateral proteinuria. During the 15 weeks of nephrosis, untreated rats developed considerable FGS (50±30), that was reduced by lisinopril (3±5, \( P = 0.058 \)) (Table 1). In untreated rats, marked focal interstitial damage was observed, as evidenced by dilated tubular segments. In ACE inhibitor-treated rats, the overall extent of tubular damage was less severe. Representative figures of histology are presented in Figure 1.

Unilateral proteinuria. FGS was elevated in the proteinuric vs the non-proteinuric kidney after 12 weeks (27±12 vs 4±5, \( P < 0.05 \)) as well as after 30 weeks (84±41 vs 33±15, \( P < 0.05 \)) of unilateral proteinuria. After 30 weeks, FGS in the non-proteinuric kidney was also elevated compared with the saline control kidney (9±8, \( P < 0.05 \)), indicating subtle damage in the non-proteinuric kidney (Table 2). Focal tubular dilatation was clearly observed in the proteinuric kidney. In contrast, in the contralateral kidney, dilated tubuli were observed only occasionally. Representative figures of histology are presented in Figure 2.

### Table 2. Results of experiment II: unilateral adriamycin-induced proteinuria

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Week 12</th>
<th>Week 30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 3)</td>
<td>AN (n = 6)</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>505±6</td>
<td>445±49</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>134±8</td>
<td>139±15</td>
</tr>
<tr>
<td>Proteinuria (mg/24 h)</td>
<td>26±14</td>
<td>261±105a</td>
</tr>
<tr>
<td>Serum creatinine (mmol/l)</td>
<td>52±12</td>
<td>62±14</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>1.87±0.63</td>
<td>3.16±1.45</td>
</tr>
<tr>
<td>Pentosidine/valine (μmol/mol)</td>
<td>0.13±0.02</td>
<td>0.16±0.06</td>
</tr>
</tbody>
</table>

Parameters at the end of the study are shown. All values are expressed as mean±SD. Focal glomerulosclerosis is expressed in arbitrary units.

AN = adriamycin nephrosis; L = left kidney (non-proteinuric); R = right kidney (proteinuric).

\( aP < 0.05 \) vs control week 12; \( bP < 0.01 \) vs control week 30; \( cP < 0.05 \) vs control week 30; \( dP < 0.01 \) vs AN week 12; \( eP < 0.05 \) vs AN left kidney week 12; \( fP < 0.01 \) vs control left kidney week 30; \( gP < 0.05 \) vs AN left kidney week 30; \( hP < 0.01 \) vs control right kidney week 30.
Immunohistochemistry

Bilateral proteinuria. In untreated adriamycin nephrosis, pentosidine staining was present in the brush border and cytoplasm of dilated tubular structures (Figure 3B–D), i.e. at sites of proteinuria-induced tubular damage. In ACE inhibitor-treated rats, the overall extent of tubular damage was less severe. However, some affected tubuli were present, and in those tubuli pentosidine was present in the brush border and cytoplasm (Figure 3D). In all groups, pentosidine staining was consistently present in collecting ducts, without differences between normal and diseased kidneys (Figure 3A and D).

Unilateral proteinuria. Pentosidine was found in the brush border and cytoplasm of dilated tubular structures in the proteinuric kidney. Consistent with the scarcity of tubular damage in non-proteinuric kidneys compared with the proteinuric kidneys, less total immunoreactive tubular pentosidine was found (Figure 4). Furthermore, pentosidine staining was consistently present in collecting ducts of all groups, without differences between normal and diseased kidneys (Figure 4).

Renal pentosidine content

Bilateral proteinuria. Renal pentosidine was elevated in untreated bilateral proteinuria (0.14 ± 0.04 μmol/mol valine) vs healthy control (0.04 ± 0.01 μmol/mol valine, P < 0.01). In lisinopril-treated rats, pentosidine content was significantly lower than in untreated rats (0.09 ± 0.02 μmol/mol valine; P < 0.05) (Table 1, individual values are given in Figure 5).

Unilateral proteinuria. No differences in renal pentosidine were seen between the non-proteinuric and proteinuric kidney, either at 12 weeks or at 30 weeks of unilateral proteinuria (Table 2, individual values are given in Figure 6). After 30 weeks, pentosidine content in adriamycin nephrotic rats was increased (0.26 ± 0.10 μmol/mol valine) compared with saline controls (0.18 ± 0.06 μmol/mol valine, P < 0.05). Pentosidine (adriamycin nephropathy, week 30) was also increased...
Fig. 3. Immunohistochemical analysis of pentosidine in experiment I (bilateral proteinuria). Magnifications are 200×. (A) Representative photograph of a section from a healthy control kidney. Accumulation of pentosidine was found in the collecting ducts (arrowheads). (B) Representative example of a section from a bilateral proteinuric rat. Accumulation of pentosidine was found in the brush border and cytoplasm of dilated tubular structures (arrows). (C) Higher magnification of a part of (B) (400×). (D) Representative photograph of a section from a bilateral proteinuric rat treated with an ACE inhibitor. The overall extent of tubular damage was less severe, i.e. less tubuli were affected, but those affected did show pentosidine at the luminal site of the tubular cell but also in the cytoplasm (arrow). Collecting ducts stained positive for pentosidine as well (arrowhead). (E) Control sections incubated with anti-pentosidine antibody solutions, which were pre-incubated with pentosidine-BSA, are negative.
compared with adriamycin nephropathy at week 12 (0.16 ± 0.06 μmol/mol valine, \( P < 0.01 \)). Although pentosidine was increased in controls at week 30 (0.18 ± 0.06 μmol/mol valine) compared with week 12 (0.13 ± 0.02 μmol/mol valine), this did not reach statistical significance due to the small numbers of rats in this group (Table 2, individual values are given in Figure 7).

**Associations across groups**

To illustrate the association between renal pentosidine and blood pressure, proteinuria, cholesterol and FGS, individual data on pentosidine accumulation in relation to the different parameters of renal damage are given in Figure 8. For animals from the different groups of experiment I pooled together, significant associations were found between urinary protein excretion and renal pentosidine accumulation (\( R^2 = 0.61, P < 0.01 \)), between cholesterol and renal pentosidine formation (\( R^2 = 0.59, P < 0.01 \)) and between histological score and renal pentosidine accumulation (\( R^2 = 0.59, P < 0.01 \)). No significant association was found between blood pressure and accumulation of pentosidine in the kidney (\( R^2 = 0.28, \text{NS} \)). It should be
noted, however, that no strict quantitative inferences should be made from the correlation coefficients as there is a clear-cut group effect.

Discussion

The present data demonstrate for the first time that renal accumulation of the AGE pentosidine occurs in non-diabetic proteinuria-induced renal damage in the experimental model of adriamycin nephrosis. On immunohistochemistry, pentosidine was localized in the brush border and cytoplasm of dilated tubular structures, i.e. at sites of proteinuria-induced renal damage. This suggests a relationship between abnormal renal protein trafficking, proteinuria-induced tubular damage and renal pentosidine accumulation, without, however, allowing a dissection between cause and consequence.

As usual in this model [13], bilateral adriamycin exposure led to the development of FGS and nephrotic-range proteinuria with overt systemic nephrosis, as apparent from the severely elevated cholesterol. In this condition, renal pentosidine was elevated. Consistent with previous experiments [12], ACE inhibition induced a marked decrease in proteinuria. The therapeutic efficacy in fact exceeds the usual therapy response in man, presumably because of the strictly

![Figure 6](https://academic.oup.com/ndt/article-abstract/20/10/2060/1934372)

Fig. 6. Renal pentosidine content in the proteinuric vs the non-proteinuric kidney (n=6 per group); data shown are week 30.

![Figure 7](https://academic.oup.com/ndt/article-abstract/20/10/2060/1934372)

Fig. 7. Renal pentosidine content in control kidneys (n=6) and adriamycin nephrotic kidneys (AN, n=12) at both week 12 and week 30.
controlled dietary sodium intake, which optimizes the antiproteinuric response [12]. This highly effective response to ACE inhibition was associated with significant amelioration of pentosidine accumulation. The immunohistochemistry data showed that pentosidine staining was present in the brush border and cytoplasm of dilated tubular structures, whereas in ACE inhibitor-treated rats fewer tubuli were affected and less pentosidine staining was observed. Together, these data demonstrate that proteinuric renal damage is associated with renal pentosidine accumulation, without, however allowing a dissection between cause and consequence.

To assess the possible role of abnormal intrarenal protein trafficking in the renal accumulation of pentosidine, we implemented unilateral proteinuric rats as well. As anticipated from prior studies, in this model there were striking differences in the extent of renal damage between the proteinuric kidney and the non-proteinuric kidney at both time points investigated. On immunohistochemistry, in accord with the findings in bilateral proteinuria, pentosidine was found in dilated tubular segments in the proteinuric kidney, i.e. at sites of proteinuria-induced renal damage. However, in the contralateral kidney, tubular pentosidine was detected only sparsely, supporting the association between abnormal renal protein trafficking, proteinuria-induced tubular damage and renal pentosidine accumulation. The immunohistochemical differences between the proteinuric and non-proteinuric kidney were not matched by a corresponding difference in cortical homogenates. However, the accumulation of tubular pentosidine was focal, with small amounts of pentosidine in damaged tubuli only, against a background of the normal presence of pentosidine in collecting ducts. This presumably explains why the differences observed by immunohistochemistry could not be detected in cortical homogenates. Moreover, as pointed out by Alderson et al. [5], tubular AGEs observed with immunohistochemistry are in the form of low molecular weight products and could be lost during the preparation of the tissue for chemical analysis.

After 30 weeks, renal pentosidine in unilateral adriamycin nephrosis and controls was increased compared with week 12, although this did not reach statistical significance in controls due to the small numbers of rats in this group. This provides evidence for age-related renal pentosidine accumulation, and suggests that the combination of non-diabetic renal damage and ageing may accelerate this phenomenon, which would be in line with data on diabetic nephropathy [16].

Could mechanisms other than intrarenal protein trafficking be involved in the renal accumulation of pentosidine? We found a time-dependent accumulation
Renal accumulation of pentosidine

of pentosidine related to renal disease. Systemic factors, due to the uremic–nephrotic milieu, might play a role in renal pentosidine deposition as well, and to analyse for such factors we included the unilateral proteinuric rats. However, the systemic changes in our unilateral rats were very mild and fell short of reaching statistical significance, so the possible contribution of systemic factors cannot validly be dissected by our data. In our bilateral proteinuric rats, on the other hand, clear-cut nephrotic hyperlipidaemia was present. Lipids as such are known to be an important source of chemical modifications and cross-links in tissue proteins [7] and thus might play a role in renal AGE accumulation. In our unilateral proteinuric rats, pentosidine accumulation occurred in the presence of mild hyperlipidaemia, which did not reach statistical significance. Apparently, prominent hyperlipidaemia is not a prerequisite for pentosidine accumulation to occur. Another factor that could be involved in the elevated renal AGES in our non-diabetic model of renal damage is increased AGE generation by oxidative stress [6], which is known to occur in renal disease and might account for carbonyl stress, leading to the production of AGES in uremia [6]. Decreased renal clearance could also contribute to renal pentosidine accumulation. Under physiological conditions, pentosidine is freely filtered by renal glomeruli, reabsorbed in the proximal tubule where it is degraded or modified, and eventually excreted in the urine [8]. Since the kidney plays a key role in pentosidine disposal, tubular dysfunction in adriamycin nephrosis can be a factor in renal pentosidine accumulation. This is supported by pentosidine accumulation in the brush border of dilated tubules, i.e. at sites of proteinuria-induced damage, which suggests impaired local degradation of AGES. Our proteinuric rats had no clear-cut reduction in renal function, as apparent from creatinine levels, indicating normal glomerular function. Preliminary data in diabetic patients suggest that a significant reduction in renal AGE clearance precedes the onset of a decline in glomerular filtration rate [17], which is consistent with reduced tubular clearance as an early mechanism.

Renoprotective treatment by lisinopril significantly ameliorated proteinuria, the systemic nephrosis and hypertension, together with amelioration in renal pentosidine accumulation. We found an association across the groups between renal pentosidine accumulation and urinary protein excretion, cholesterol and FGS. However, no such association was found between blood pressure and accumulation of pentosidine in the kidney. The reduction in renal pentosidine by treatment with an ACE inhibitor instead of by ACE inhibitor treatment may thus have been secondary to the renoprotective, antiproteinuric effect, possibly also including its concomitant effect on cholesterol. Alternatively, direct effects of the ACE inhibitor on renal AGE accumulation may have been involved, as ACE inhibitors were shown to affect the production of AGE precursors by chelating transition metal ions, which catalyse the formation of AGES in the Maillard reaction, and inhibiting various oxidative steps in the process of glycoxidation, including the formation of free radicals [18]. It should be noted, however, that our study does not allow conclusions on the mechanism by which lisinopril reduced renal AGE accumulation and that further studies would be needed to address this question. Our data are in line with data showing that treatment with the angiotensin II receptor blocker losartan improved renal function and ameliorated the rise in serum AGES in normotensive subtotally nephrectomized rats, which was independent of blood pressure [19]. Interestingly, blocking both the renin–angiotensin and advanced glycation pathways was found to offer superior renoprotection compared with either treatment alone [20].

What could be the pathophysiological significance of renal pentosidine accumulation? Several studies support the in vivo [3] and in vitro nephrotoxicity of AGES [4]. As reviewed recently, AGE-modified proteins initiate a range of cellular responses including enhanced growth factor expression, cellular proliferation and apoptosis, angiogenesis and tissue remodelling [10] by binding to AGE-specific receptors [1], that might be involved in nephrotoxicity. RAGE, a receptor for AGES, contributes to mesangial activation and transforming growth factor-β production; processes which converge to cause albuminuria and glomerulosclerosis [21]. These data suggest that renal AGE accumulation that has resulted from a primary renal disorder, once present, can itself become a perpetuating factor in ongoing renal damage. Our present data, however, do not allow such a conclusion, as this would require specific intervention aimed at reduction of AGE accumulation, such as inhibition of AGE formation or stimulation of AGE breakdown.

In conclusion, renal pentosidine accumulation occurs in non-diabetic proteinuric kidneys. The accumulation occurs in damaged tubules, and is ameliorated by renoprotective treatment by ACE inhibition, supporting a relationship between abnormal renal protein trafficking, proteinuria-induced tubular damage and tubular pentosidine accumulation. Future studies, applying specific AGE inhibitors, should be conducted to yield insight into the pathophysiological significance of renal AGE accumulation in the progression of non-diabetic renal disease.

Acknowledgements. This study was funded by a grant from the JK de Cock Stichting and by a grant from the US National Institutes of Diabetes & Digestive & Kidney Diseases (DK-19971). The authors would like to thank Marian Bulthuis for her skilled technical assistance.

Conflict of interest statement. None declared.

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Received for publication: 24.8.04
Accepted in revised form: 11.5.05