Proteinuria-associated renal injury and the effects of intervention in the renin-angiotensin-aldosterone system
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2006

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Chapter 4

De novo induction of Kidney injury molecule-1 (Kim-1) in proteinuria-induced renal damage

Andrea Kramer, Mirjan van Timmeren, Theo Schuurs, Vishal Vaidya, Joseph Bonventre, Harry van Goor and Gerjan Navis
ABSTRACT
Tubulointerstitial lesions are important in the progression of proteinuric renal disease. Tubular Kim-1 is induced after acute renal injury. The role of Kim-1 in chronic proteinuria-induced renal damage and effects of antiproteinuric treatment on Kim-1 are unknown. We studied Kim-1 in adriamycin nephrosis before and after treatment with RAAS-blockade. A renal biopsy was taken 6 weeks after adriamycin injection to study renal damage and Kim-1 expression. Subsequently, ACE-inhibition (ACEi, n=23), angiotensin II type 1 -antagonist (AT1A, n=23) or vehicle (n=10) was given for 6 weeks, with healthy rats as controls (n=8). We measured Kim-1 mRNA (qPCR), protein (immunohistochemistry) and urinary Kim-1 (microfluidics). In proteinuric rats renal Kim-1 mRNA was induced 26-fold vs controls at week 6, with a further increase in vehicle (40-fold, week 12), but a reduction by ACEi and AT1A to 10- and 12-fold vs control (p<0.05 vs week 6). Kim-1 protein was undetectable in control kidneys; in proteinuric kidneys it was present in the brush border of dilated tubules in areas with adjacent interstitial lesions. Renal Kim-1 protein levels increased from week 6 to 12 in vehicle and decreased in ACEi and AT1A-treated groups (p<0.05). In vehicle-treated rats urinary Kim-1 (week 12) was increased (p<0.05 vs controls), with a reduction by ACEi and AT1A (p<0.05 vs vehicle). Renal and urinary Kim-1 correlated with proteinuria and interstitial damage. In conclusion, Kim-1 is induced in chronic proteinuria-induced renal damage, associated with proteinuria and interstitial injury and reduced by antiproteinuric treatment. Further studies should elucidate its functional role in proteinuria-induced interstitial fibrosis.
INTRODUCTION

Long-term proteinuria causes tubulointerstitial damage, which slowly progresses to end stage renal disease. These tubulointerstitial lesions can predict the clinical course [1-4] and the efficacy of antiproteinuric treatment [5;6] in patients. Intervention in the renin-angiotensin aldosterone system (RAAS) provides renoprotection by reducing blood pressure, proteinuria and renal structural damage [7;8]. By reducing proteinuria RAAS-blockade might protect for tubular injury.

Kidney injury molecule-1 is a recently discovered type 1 membrane protein that is expressed at negligible levels in normal rat kidneys, but is massively induced in tubules after ischemic or toxic injury in rats [9;10]. In these rats Kim-1 expression is localized to proximal tubular cells (PTC) many of which display characteristics of injury and regeneration, as evidenced by loss of brush border, a flat cell structure and luminal debris [9-11]. The Kim-1 protein has a short cytoplasmic domain and extracellular Ig and mucin domains [9], which can be shed into urine. Urinary Kim-1 levels are associated with Kim-1 protein expression in injured nephrons [10]. Recently, KIM-1 was shown to be a new urinary biomarker for the early detection of renal cell carcinoma [12], to be associated with epithelial dedifferentiation and interstitial fibrosis in murine polycystic kidney disease [11], and with chronic proteinuria-induced renal damage in protein-overload nephropathy [13]. Together, these data suggest that Kim-1 is not specific for acute and ischemic renal disease, but can be involved in various forms of tubulointerstitial damage, including chronic conditions.

In acute renal damage Kim-1 induction can be reversible, corresponding to the natural course of the disease with spontaneous recovery [10]. The dynamics of Kim-1 expression in chronic renal damage are less well characterized, neither with respect to the natural course of progressive renal damage, nor with respect to possible reversibility during renoprotective intervention. Therefore, we studied Kim-1 in adriamycin-induced nephrosis, a well-characterized rat model of chronic proteinuria-induced renal damage, both during the natural course, and before and after renoprotective intervention with RAAS blockade. Parameters of interstitial inflammation and fibrosis were measured to establish a possible association with Kim-1 induction.

METHODS

Animals

Sixty-four male Wistar rats, (HsdCpb:Wu; Harlan, Zeist, The Netherlands) weighing 336±17 gram at disease induction, were studied. Rats were housed in a temperature-controlled room with a 12 hour light-dark cycle and free access to food and water. Urine was collected 2-weekly during a 24-hour period in metabolic cages and stored at -20°C.

All surgical procedures took place under isoflurane anesthesia in N₂O/oxygen (1:2). Adriamycin nephrosis (AN) was induced by injection of 2 mg/kg adriamycin (Doxorubicin®)
into the tail vein, 6 weeks thereafter a renal biopsy was performed via dorsolateral incision to study pre-treatment renal damage. Immediately after surgical removal of a small part of the lower pole from the left kidney, gelfoam was applied to achieve haemostasis. Renal tissue samples were snap-frozen in liquid nitrogen and stored at -80, another part was fixed in 4% paraformaldehyde and embedded in paraffin.

After recovery from the biopsy groups were treated with vehicle treatment (VEH, n=10), ACE inhibitor (ACEi, lisinopril 75 mg/L drinking water, equal to 5 mg/kg/day, n=23) or AT1A (L158,809 150 mg/L drinking water, equal to 10 mg/kg/day, n=23) [14]. In prior experiments we showed that the biopsy procedure does not affect the course of renal damage [15]. Treatment was continued for 6 weeks until sacrifice at week 12. Immediately after surgery for the biopsy 4 animals died in the VEH group, 1 in the ACE inhibitor group and 3 in the AT1A group; these animals were not included in the analyses. At the end of the study a 2 ml blood sample was taken by cannulation of the abdominal aorta, kidneys were perfused with saline, removed and further processed as with the biopsy. The protocol was approved by the Committee for Animal Experiments of the University of Groningen, The Netherlands.

**Clinical parameters**

Proteinuria was measured by the Biuret method (Bioquant; Merck, Darmstadt, Germany). Plasma and urine creatinine levels were determined colorometrically (Sigma, St Louis, MO, USA). Systolic blood pressure (SBP) was measured weekly by the tail cuff method in trained conscious rats [16].

**Microfluidics assay for urinary Kim-1**

Urinary Kim-1 was measured by microsphere-based Luminex xMAP™ technology [17]. This technique is an adaptation of the recently developed and validated sandwich ELISA assay [18].

**Quantitative PCR for Kim-1**

Total RNA was isolated using an adaptation of the standard guanidine thiocyanate lysis [19]. First-strand cDNA was synthesized from 1 µg total RNA (RT-PCR Core kit, Perkin Elmer, USA). TaqMan real-time PCR was performed in 386-wells plates on an ABI Prism 7900 Sequence Detector System (Applied Biosystems, Foster City, CA, USA) with SYBR green. Each reaction well contained 5 µl cDNA, forward and reverse primers 50 µM (0.4 µl each), 10 µl SYBR Green master mix and 4.2 µl nuclease-free water. The PCR plate was incubated for 2 minutes at 50°C, 10 min at 95°C, followed by 40 cycles with 15 sec at 95°C and 1 min at 60°C. All assays were performed in triplicate with non-template controls, and the coefficient of variation was < 5% for all replicates. The C<sub>T</sub> (threshold cycle) is inversely proportional to the logarithmic scale of the starting quantity of template cDNA. The C<sub>T</sub> values for Kim-1 were subtracted from the housekeeping gene (ß-actin) C<sub>T</sub> value to yield the ΔC<sub>T</sub>. The results were finally expressed as 2-ΔΔC<sub>T</sub>, an index of the relative amount of Kim-1 mRNA in each sample. Primer sequences are given in Table 1.
**Table 1. Primer sequences for qPCR and in-situ hybridisation**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Forward</th>
<th>Reverse</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kim-1 for PCR</td>
<td>5' AGA GAG AGC AGG ACA CAG GCT TT 3'</td>
<td>5' ACC CGT GGT AGT CCC AAA CA 3'</td>
<td>75 bp</td>
</tr>
<tr>
<td>β-actin for qPCR</td>
<td>5' GGA AAT CTG GCG TGA CAT TAA A 3'</td>
<td>5' GCG GCA GTG GCC ATC TC 3'</td>
<td>74 bp</td>
</tr>
<tr>
<td>Kim-1 for ISH</td>
<td>5' -AAC GCA GCG ATT GTG CAT CC-3'</td>
<td>5' -GTC CAC TCA CCA TGG TAA CC-3'</td>
<td>696 bp</td>
</tr>
</tbody>
</table>

**In-situ hybridization (ISH)**

The 696 bp Kim-1 PCR product was subcloned into the pCRII-TOPO vector (Invitrogen, Carlsbad, USA). RNA probes were labeled with a DIG RNA labelling kit (Sp6/T7, Roche, Mannheim, Germany). ISH was performed on routinely fixed paraffin-embedded tissue sections using standard laboratory protocols. Briefly, deparaffinized sections were air-dried, treated with Triton X-100, followed by proteinase K. Thereafter, slides were incubated with DIG-labeled probe in a hybridization solution consisting of 1 ml 20x SSC, 50 µl 100x Denhardt’s solution, 1 ml 50% Dextran sulphate, 2.5 ml formamide, 200 µl t-RNA, 50 µl 1M DTT and 125 µl salmon sperm DNA overnight at 55°C. After washing, slides were treated with 2 U/ml RNase T1 in 1 mM EDTA and 2x SSC at 37°C for 30 minutes. Positive cells were visualized with anti-DIG labeled alkaline-phosphatase for one hour at 37°C in 0.1M maleic acid buffer containing 0.15M NaCl, 1% blocking buffer and 2% normal sheep serum. Staining reaction was performed for 48 hours at 4°C with NBT and BCIP in TBS with MgCl₂ and levamisole.

**Immunohistochemistry**

Paraffin sections (4 µm) were stained with periodic acid-Schiff (PAS) to evaluate focal glomerulosclerosis (FGS) and interstitial fibrosis (IF). Immunostaining was performed on paraffin sections for Kim-1 (antibody against the intracellular domain of Kim-1: peptide 9, dilution 1:1000, Biogen, Cambridge, MA, USA), α-smooth muscle actin (α-SMA, clone 1A4, dilution 1:15,000, Sigma, St. Louis, MO, USA), collagen type III (dilution 1:100, Biogenesis, Poole, UK) and macrophages (ED1, dilution 1:1000, Serotec, Oxford, UK). After dewaxing with xylol and alcohol, antigen-retrieval was performed by overnight incubation (80°C) in 0.1M TRIS/HCl buffer. After blocking of endogenous peroxidase-activity, sections were incubated for 1 hour with diluted primary antibodies in PBS with 1% bovine serum albumin. Binding for antibodies was detected using two sequential incubations (30 min) with peroxidase-labeled secondary antibodies. Peroxidase activity was developed using 3,3’-diamino benzidine (DAB, Sigma) solution for 10 min, to which hydrogen peroxide was added. An automated staining system (DAKO Autostainer, Edition 4.0, DAKO Corporation, Carpinteria, CA, USA) was used to obtain comparable staining results for all slides.
To study the co-localization of Kim-1 with renal interstitial damage, double staining with ED1 (macrophages), α-SMA and collagen III was performed. Slides were incubated with a mixture of primary antibodies for 1 hour at room temperature, anti-Kim-1 and anti-ED1 at dilution 1:400, α-SMA at 1:500. After washing with PBS secondary antibodies were added. Kim-1 was detected with peroxidase-labeled goat anti-rabbit antibodies and ED1 and α-SMA with alkaline-phosphatase-labeled goat anti-mouse antibodies. First, peroxidase activity was developed with DAB for 10 min. Subsequently, alkaline-phosphatase activity was developed with Naphtol AS-MX and colour developed with Fast Blue BB combined with levamisol and MgSO$_4$ during 30 min. To combine collagen III and Kim-1 (both polyclonal), staining for Kim-1 with peroxidase-labeled secondary antibodies and DAB was first performed, followed by incubation with glycine/HCl pH 2.0 for 30 min. After blocking of endogenous biotin and streptavidin sections were incubated with anti-collagen III (dilution 1:50) followed by a biotin-labeled goat anti-rabbit antibody and alkaline-phosphatase-labeled streptavidin. Appropriate isotype and PBS controls were consistently negative for all antibodies.

**Quantification of renal damage**

FGS was scored semi-quantitatively on a scale 0 to 4 in 50 glomeruli per kidney moving from outer to inner cortex. FGS lesions were defined as glomerular areas with mesangial expansion and adhesion formation simultaneously present in one segment [20]. Interstitial fibrosis was scored semi-quantitatively on a scale of 0-3 [21]. Interstitial Kim-1, α-SMA and collagen type III staining were measured by a blinded observer using computer image analysis (Advanced QUIPS, Leica Imaging Systems, Cambridge, UK). The proportional area of immunostaining was measured in 50 randomly selected cortical interstitial images per kidney, with exclusion of large vessels and glomeruli. The area of immunostaining for Kim-1, α-SMA and collagen III was divided by the total surface of the image. The number of glomerular macrophages was determined in 50 glomeruli per slide. Interstitial macrophages were counted in 50 consecutive fields.

**Statistical analysis**

Data are expressed as mean and standard deviation. Data distribution was not normal; therefore, non-parametric tests were used. For differences between the treatment groups, a Kruskal Wallis test was used. For differences between week 6 and 12 the Wilcoxon Signed Rank test was used. Spearman’s Rho correlation coefficients are given. To perform linear regression data were transformed with natural logarithm (LN). Statistical analyses were performed using SPSS statistical software version 12.0 and GraphPad Prism version 3.02. Statistical significance was assumed at the 5% level.
RESULTS

Clinical parameters
At week 6, overt proteinuria was present in the adriamycin rats. During ACEi treatment proteinuria decreased by 78±15% and with AT1A by 59±24% (both p<0.05 vs adriamycin at week 6). In the VEH animals, proteinuria stabilized from 6 week onwards (Fig. 2A). Blood pressure was significantly reduced by ACEi and by AT1A but was stable during VEH. Compared to healthy controls, creatinine clearance was significantly reduced in all adriamycin groups (Table 2).

<table>
<thead>
<tr>
<th>Blood pressure (mmHg)</th>
<th>Week</th>
<th>VEH (n=6)</th>
<th>ACEi (n=22)</th>
<th>AT1A (n=21)</th>
<th>CON (n=8)</th>
</tr>
</thead>
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<tr>
<td>6</td>
<td>151±11</td>
<td>146±9</td>
<td>144±11</td>
<td>123±17</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>139±24</td>
<td>103±20*#</td>
<td>118±23*</td>
<td>118±14</td>
<td></td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>12</td>
<td>1.37±0.88* &amp; 1.29±0.39*</td>
<td>1.43±0.37*</td>
<td>2.42±0.82</td>
<td></td>
</tr>
<tr>
<td>Macrophages (per interstitial field)</td>
<td>6</td>
<td>136±103</td>
<td>94±64</td>
<td>88±58</td>
<td></td>
</tr>
<tr>
<td>α-SMA (% interstitial staining)</td>
<td>6</td>
<td>8.1±2.8</td>
<td>7.2±3.1</td>
<td>7.0±3.4</td>
<td></td>
</tr>
<tr>
<td>Collagen type III (% interstitial staining)</td>
<td>6</td>
<td>0.42±0.21</td>
<td>0.54±0.21</td>
<td>0.58±0.31</td>
<td></td>
</tr>
<tr>
<td>Interstitial fibrosis (Score 0-3)</td>
<td>6</td>
<td>0.5±0.8</td>
<td>0.9±1.0</td>
<td>0.6±0.7</td>
<td></td>
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<tr>
<td>FGS (Score 0-400)</td>
<td>6</td>
<td>17±11</td>
<td>41±41</td>
<td>20±27</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>103±55*#</td>
<td>47±46*#</td>
<td>56±72*#</td>
<td>6±6</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Clinical parameters and renal damage

Data are expressed as mean ± standard deviation; * p<0.05 vs week 6, #p<0.05 vs VEH, & p<0.05 vs CON

Quantification of renal damage
Macrophage influx was high in adriamycin rats at week 6 and decreased in all groups from week 6 to 12. In adriamycin rats α-SMA expression was present at week 6. It was increased compared to control rats and stabilized during treatment in all groups. Collagen type III staining and interstitial fibrosis were increased in adriamycin rats at week 12 compared to healthy controls, without an apparent effect of ACEi and AT1A. Focal glomerulosclerosis was present at week 6; treatment with ACEi and AT1A attenuated FGS at week 12, whereas FGS was further increased in VEH by that time (Table 2).
Figure 1. Localization of Kim-1 In situ hybridization (A+B) and immunohistochemistry (C-F) for rat Kim-1. A) Kim-1 mRNA in a healthy control rat, no Kim-1 mRNA is visible (magnification 100x). B) Kim-1 mRNA is induced in rats with adriamycin nephrosis (AN) in dilated proximal tubular epithelial cells. Several tubules, most probably adhering to one nephron, are positive for Kim-1 mRNA (400x). C) Kim-1 protein expression in a rat with AN at week 6 (100x). Several tubules, which seem to adhere to one nephron, are positive for Kim-1. Kim-1 is expressed in the dilated proximal tubules at the apical membrane, which is shown in more detail in D) (400x). Here it is also visible that Kim-1 shows a mosaic pattern, not all cells are positive for Kim-1. E) Kim-1 protein expression in a VEH treated rat at wk 12, expression of Kim-1 was somewhat higher than at week 6. F) In healthy control rats at week 12, no Kim-1 protein was detected. G) After 6 weeks of ACEi treatment Kim-1 protein was reduced, but in dilated tubules still some Kim-1 expression was visible. H) Treatment with AT1A also reduced Kim-1 staining.
RAAS blockade reduces Kim-1 induction

**Figure 2.** Bar graphs of proteinuria (A), Kim-1 mRNA measured with quantitative real time PCR (B) and Kim-1 protein measured by computer-assisted morphometry (C) in the different treatment groups. Abbreviations: VEH: vehicle treatment ACEi: angiotensin-converting enzyme inhibition; AT1A: angiotensin type 1 antagonist; CON: healthy control rats.

**Figure 3.** Double staining of Kim-1 in combination with macrophages, α-smooth muscle lesions. In later stages macrophages are widespread present in the whole cortex. 

A) Double staining for Kim-1 (brown) and α-smooth muscle actin (blue). Kim-1 positive dilated tubules are often surrounded by myofibroblasts (arrows). 

B) Double staining for Kim-1 (brown) and α-smooth muscle actin (blue). Kim-1 positive dilated tubules are often surrounded by myofibroblasts (arrows). 

C) Double staining for Kim-1 (brown) and collagen type III (blue). Flattened tubules that are positive for Kim-1 are sometimes surrounded by increased deposition of collagen type III (arrow). However, in advanced stages, also many dilated tubules surrounded by collagen III deposition are negative for Kim-1.

**Kim-1 qPCR**

The pre-treatment biopsies from week 6 of all adriamycin animals showed a massive induction of Kim-1 mRNA ranging from 18- to 30-fold induction (adriamycin vs CON: p<0.05). At the end of the study Kim-1 mRNA expression was increased to a 40-fold induction in VEH rats, whereas
antiproteinuric treatment reduced Kim-1 mRNA expression to a 10-fold induction in ACEi, and 12-fold in AT1A treated rats (both p<0.05 vs VEH) (Fig. 2B).

**Kim-1 in-situ hybridisation**

In healthy rats, no renal Kim-1 mRNA was detected (Fig. 1A). In adriamycin rats Kim-1 mRNA was clearly present and mainly localized in the cytoplasm of tubular cells from focally grouped dilated tubules (Fig. 1B). However, in the most severely damaged tubules - characterized by advanced dilation and severe flattening of tubular cells- Kim-1 mRNA was not easily seen. Single nephrons could often be traced from cortex to medulla by their positive Kim-1 mRNA staining.

**Kim-1 immunohistochemistry**

In concert with the expression pattern observed with in situ hybridization Kim-1 protein positive tubules in adriamycin rats were often organized in segments suggestive for the contours of a single nephron (Fig. 2C). Kim-1 protein was prominent at the apical border of dilated tubular cells with weak cytoplasmic expression. Kim-1 was often present in slightly dilated tubules, with morphologically well-differentiated cells. In more dilated tubules, Kim-1 expression within one tubule showed a mosaic staining pattern with positive and negative cells adjacent to each other (Fig. 2D). In severely damaged tubules, as apparent from advanced flattening of the tubular cells, Kim-1 was absent (Fig. 2E). Kim-1 expression was absent in the glomerulus, peritubular interstitial cells and inner medullary cells. Computerized quantification revealed that Kim-1 protein expression was similar at week 6 (before start of treatment) for all groups (Fig. 1C and 2C). At week 12 Kim-1 protein was significantly reduced in the ACEi and AT1A groups (from 0.23±0.25 to 0.10±0.10 and 0.23±0.16 to 0.13±0.10 % of tissue volume respectively, comparing week 6 to 12, both p<0.05) (Fig. 2F and G). In the VEH group Kim-1 protein increased (from 0.23±0.20 to 0.35±0.23 % of tissue volume, comparing week 6 to 12, p<0.05, Fig. 2E). In healthy controls almost no Kim-1 protein was present (0.01±0.01 % tissue volume, Fig. 2H).

**Co-localization studies**

To associate renal Kim-1 expression with classical markers of tubulo-interstitial damage we performed double staining for Kim-1 with a macrophage marker (ED1, inflammation), α-SMA expression (myofibroblast transformation, indicating the presence of pre-fibrotic changes) and collagen type III (fibrotic lesions). The double staining of Kim-1 with ED1 showed that Kim-1 positive tubules were often surrounded by interstitial macrophages (Fig. 3A), whereas Kim-1 negative (and morphologically normal) tubules were not associated with macrophage infiltration. However, in more advanced fibrotic lesions, macrophages were also present in areas with severely dilated Kim-1 negative tubules. The Kim-1 positive tubules were surrounded by α-SMA positive fibroblasts, indicating the presence of pre-fibrotic changes (Fig. 3B). In this double staining, also severely dilated, but Kim-1 negative, tubules were surrounded by α-SMA positive
fibroblasts. Kim-1 positive tubules were often surrounded by collagen III deposits (Fig. 3D). However, this co-localization was not always present, especially not in advanced lesions.

**Urinary Kim-1**

The shedded ectodomain of Kim-1 in urine was measured by a microfluidics (Luminex R) based assay at the end of the study. The concentration of urinary Kim-1 was significantly increased in all adriamycin animals at week 12. In the VEH-treated group urinary Kim-1 was 257±164 pg/mL, versus 21±10 pg/mL in controls (p<0.05). Treatment with ACEi or AT1A for 6 weeks significantly reduced urinary Kim-1 to 53±47 and 91±111 pg/mL respectively (both p<0.01 vs VEH).

![Figure 4. Scatter plot showing the strong correlation between urinary and renal Kim-1 expression. Data were transformed with the natural logarithm to obtain a normal distribution.](image)

**Correlations between urinary and renal Kim-1, proteinuria and renal damage**

On individual analysis urinary Kim-1 and renal Kim-1 were strongly associated (r=0.67, p<0.001, Fig. 4). For the parameters separately, renal Kim-1 (mRNA) was strongly associated with proteinuria (r=0.65, p<0.001, Fig. 5A) and with markers of renal damage. The association with α-SMA is high (r=0.72, p<0.001, Fig. 5C). Kim-1 mRNA was also associated with other parameters of renal damage: tissue macrophages (r=0.39, p<0.05), interstitial fibrosis (r=0.66, p<0.01), FGS (r=0.70, p<0.01). Also, urinary Kim-1 is associated with proteinuria (r=0.74, p<0.001, Fig. 5B) and with renal damage: α-SMA (r=0.57, p<0.001, Fig. 5D), macrophages (r=0.48, p<0.01), interstitial fibrosis (r=0.55, p<0.01) and FGS (r=0.74, p<0.01). Thus, on cross-sectional analysis, animals with more proteinuria have higher levels of renal Kim-1 and urinary Kim-1. Higher levels of Kim-1 mRNA and urine are also associated with more renal damage.
DISCUSSION

The major findings of this paper are the strong induction of Kim-1 in a model of chronic proteinuria-induced renal damage (adriamycin nephrosis), and the reduced expression of renal tubular Kim-1 after antiproteinuric treatment by RAAS blockade. Kim-1 expression was present in injured and dilated proximal tubules surrounded by interstitial fibrosis and inflammation. We also showed that in experimental nephrosis, tubular Kim-1 is shed and present in the urine. For individual rats the levels of urinary Kim-1 were strongly associated with renal tissue Kim-1 and proteinuria.

This study is, to our knowledge, the first to describe the induction and reversibility of Kim-1 in proteinuria-induced renal damage. Adriamycin-induced proteinuria is a standardized model of proteinuria-induced renal damage characterized by the development of proteinuria a few days after injection of adriamycin, which stabilizes after 4 to 5 weeks [22]. Persistent proteinuria is thought to induce interstitial attraction of inflammatory cells, which subsequently initiate the development of interstitial fibrosis [23]. Tubular activation by the proteinuric ultrafiltrate is known to induce a chemotactic response in vitro [24]. This model is therefore
particularly suitable to study the mechanisms of proteinuria induced tubular damage, and the effects of antiproteinuric treatment on the reversibility of tubular expression of markers of damage.

The mechanism of Kim-1 induction was not specifically studied here, but several options should be considered. Kim-1 could be induced by proteinuria as such by tubular reabsorption of leaked proteins, or by proteinuria-induced growth factors and cytokines [24], leading to proliferation, apoptosis, inflammation and increased extracellular matrix production. This possibility is supported by our recent finding that Kim-1 is induced in protein-overload induced proteinuria as well [13]. Kim-1 could also be induced by hypoxic conditions, which might occur due to loss of peritubular capillaries with injury [25]. Kim-1 was originally found in ischemic renal disease.

After antiproteinuric treatment with RAS blockade the increase in renal and urinary Kim-1 was found to be reduced to a large extent. This could be secondary to reduction of proteinuria and its intrarenal sequelae, or to direct pharmacological effects of the intervention by amelioration of angiotensin II-dependent induction in the release of cytokines and growth factors [26]. Moreover, RAS blockade can improve medullary blood flow and tubular oxygenation, which may support restoration of tubular integrity [27]. In our model the reduction of proteinuria and angiotensin II appear plausible mechanisms.

We studied the intrarenal and cellular localization of Kim-1 in relation to the presence of tubulointerstitial damage. We found Kim-1 localized in the apical membrane of dilated tubules, which corresponds to the localization of Kim-1 in ischemic and toxic injury [9;10]. In ischemic injury, Kim-1 expression is most prominent in the S3 segment (i.e the segment most susceptible to ischemic injury) whereas in our model of proteinuria-induced renal damage Kim-1 expression was also prominent in the mid-cortical and superficial tubules, which is in congruence with the Kim-1 expression in folic acid-induced renal injury and polycystic kidney disease, models where damage is not predominantly in the S3 segment [10;11]. Therefore, localization of Kim-1 expression appears to be related to the susceptibility of the tubules for the different types of injury.

When related to localization of tubulointerstitial damage, we found that Kim-1 was expressed in areas that display interstitial inflammation, tubular dilatation, and (pre)-fibrotic changes. Interestingly, Kim-1 expression was mainly apparent at the apical membrane of dilated tubular cells, whereas it was absent in advanced stages of tubular damage. We observed a mosaic pattern of Kim-1 staining within one tubule: cells with preserved morphology within the tubule showed Kim-1 expression, and the more flattened cells were Kim-1 negative. In ischemia/reperfusion Kim-1 is co-localized with vimentin (dedifferentiation) and BrdU (proliferation) in regenerating tubular cells [9]. In polycystic kidney disease [11] Kim-1 positive cells demonstrate partial loss of polarity but preserved staining for actin, villin and E-cadherin. Together these data suggest that Kim-1 is expressed early in the sequence of events of
dedifferentiation of an injured tubular cell. It should be noted in this respect that we found reversibility of renal Kim-1 during treatment, but no reversibility of interstitial fibrosis, reinforcing the notion of predominant association with early tubulointerstitial changes and proteinuria. Whether, in the context of early tubular lesions, Kim-1 contributes to the progression of damage or exerts a protective role, however, cannot be derived from our data. We suggest that Kim-1 might protect tubular cells from proteins and other toxic mediators or play a role in recuperative processes of the tubules, as it is expressed in early stages and is absent in advanced stages of tubular damage. However future experiments with in vitro studies and knockout mice are necessary to elucidate the pathophysiological significance of Kim-1.

Kim-1 is shed into urine, and, in accord with other studies, we found that its urinary excretion corresponded to the extent of intrarenal Kim-1 expression. Urinary Kim-1 was increased in untreated proteinuric animals, whereas animals after antiproteinuric treatment showed lower levels of urinary Kim-1. As urinary Kim-1 correlates with proteinuria and renal damage it is a potential non-invasive marker for the extent of renal damage. Therefore, it would be of great interest to see whether the effects of treatment on urinary Kim-1 could prospectively predict renal outcome of treatment. In this study, however, we did not measure urinary Kim-1 levels before treatment and therefore we cannot draw conclusions on this issue. Whether Kim-1 has functional significance for protection or damage of the tubular brush border would be of interest, but cannot be derived from our data either.

In conclusion, Kidney injury molecule-1 is induced in chronic proteinuria-induced renal disease and is associated with proteinuria and early interstitial injury. The increase in renal and urinary Kim-1 levels is to a large extent reversible by antiproteinuric intervention with RAS blockade. The localization studies suggest that Kim-1 is associated with the early stages of dedifferentiation of tubular cells, but functional studies are needed to establish its pathophysiological significance.

References


