

ORIGINAL INVESTIGATIONS

Pathogenesis and Treatment of Kidney Disease

Effect of Renin-Angiotensin-Aldosterone System Inhibition, Dietary Sodium Restriction, and/or Diuretics on Urinary Kidney Injury Molecule 1 Excretion in Nondiabetic Proteinuric Kidney Disease: A Post Hoc Analysis of a Randomized Controlled Trial

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Background: Tubulointerstitial damage plays an important role in chronic kidney disease (CKD) with proteinuria. Urinary kidney injury molecule 1 (KIM-1) reflects tubular KIM-1 and is considered a sensitive biomarker for early tubular damage. We hypothesized that a decrease in proteinuria by using therapeutic interventions is associated with decreased urinary KIM-1 levels.

Study Design: Post hoc analysis of a randomized, double-blind, placebo-controlled, crossover trial.

Setting & Participants: 34 proteinuric patients without diabetes from our outpatient renal clinic.

Intervention: Stepwise 6-week interventions of losartan, sodium restriction (low-sodium [LS] diet), their combination, losartan plus hydrochlorothiazide (HCT), and the latter plus an LS diet.

Outcomes & Measurements: Urinary excretion of KIM-1, total protein, and *N*-acetyl- β -D-glucosaminidase (NAG) as a positive control for tubular injury.

Results: Mean baseline urine protein level was 3.8 ± 0.4 (SE) g/d, and KIM-1 level was $1,706 \pm 498$ ng/d (increased compared with healthy controls; 74 ng/d). KIM-1 level was decreased by using placebo/LS ($1,201 \pm 388$ ng/d; $P = 0.04$), losartan/high sodium ($1,184 \pm 296$ ng/d; $P = 0.09$), losartan/LS (921 ± 176 ng/d; $P = 0.008$), losartan/high sodium plus HCT (862 ± 151 ng/d; $P = 0.008$) and losartan/LS plus HCT (743 ± 170 ng/d; $P = 0.001$). The decrease in urinary KIM-1 levels paralleled the decrease in proteinuria ($R = 0.523$; $P < 0.001$), but not blood pressure or creatinine clearance. 16 patients reached target proteinuria with protein less than 1 g/d, whereas KIM-1 levels normalized in only 2 patients. Urinary NAG level was increased at baseline and significantly decreased during the treatment periods of combined losartan plus HCT only. The decrease in urinary NAG levels was not closely related to proteinuria.

Limitations: Post hoc analysis.

Conclusions: Urinary KIM-1 level was increased in patients with nondiabetic CKD with proteinuria and decreased in parallel with proteinuria by using losartan, sodium restriction, their combination, losartan plus HCT, and the latter plus sodium restriction. These results are consistent with the hypothesis of amelioration of proteinuria-induced tubular damage. Long-term studies are warranted to evaluate whether targeting treatment on KIM-1 can improve outcomes in patients with CKD with proteinuria.

Am J Kidney Dis 53:16-25. © 2008 by the National Kidney Foundation, Inc.

INDEX WORDS: Renin-angiotensin-aldosterone system; losartan; angiotensin II type 1 receptor blockade; proteinuria; interstitial renal damage; kidney injury molecule 1; *N*-acetyl- β -D-glucosaminidase; tubular damage marker; biomarker.

Editorial, p. 1

Proteinuria is a main determinant of chronic kidney disease (CKD). In this process, proteinuria-induced tubulointerstitial inflammation

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Received February 17, 2007. Accepted in revised form July 7, 2008. Originally published online as doi: 10.1053/j.ajkd.2008.07.021 on September 29, 2008.

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0272-6386/08/5301-0005\$36.00/0

doi:10.1053/j.ajkd.2008.07.021

and damage have an important pathophysiological role. The direct detrimental effect of proteins on tubular cells has been shown in vitro and in vivo. Albumin stimulates the production of, for example, monocyte chemoattractant protein 1 (encoded by the *CCL2* gene), Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES) (encoded by *CCL5*), and interleukin 8 (*IL8*) by proximal tubular cells in vitro.¹⁻⁴ In vivo, urinary tubular protein trafficking leads to activation of intracellular signaling pathways in experimental protein-overload nephropathy.⁵ The resulting increased synthesis and secretion of proinflammatory mediators attract and activate macrophages, a process leading to interstitial fibrosis and, ultimately, loss of kidney function.⁵

In patients with proteinuria-associated CKD, a decrease in proteinuria is consistently associated with improvement in long-term outcome. A decrease in proteinuria therefore is recognized as an independent treatment target for renoprotective intervention, with a target level of protein less than 1 g/d.^{6,7} A decrease in proteinuria-induced glomerular and tubulointerstitial damage is assumed to be involved in the long-term renoprotective effect of proteinuria reduction.^{7,8}

Kidney injury molecule 1 (KIM-1; encoded by *HAVCR1*) is a sensitive marker for the presence

of tubular damage.⁹ KIM-1 is not detectable in healthy kidney tissue, but is significantly induced in various human primary and secondary kidney diseases and in allograft nephropathy.¹⁰ Tubular KIM-1 expression is significantly associated with tubulointerstitial damage and inflammation.¹⁰ In experimental and human kidney disease, increased urinary KIM-1 levels are strongly related to tubular KIM-1 expression, showing that urinary KIM-1 level can be a valuable biomarker for the presence of tubulointerstitial damage.⁹⁻¹¹ Furthermore, urinary excretion of KIM-1 is an independent predictor of graft loss in kidney transplant recipients, indicating its prognostic impact.¹²

We recently showed a stepwise decrease in proteinuria by using a regimen of a low-sodium (LS) diet and losartan, 100 mg/d, individually, in combination, and in combination with hydrochlorothiazide (HCT) in a randomized, double-blind, placebo-controlled, crossover study of proteinuric patients without diabetes.¹³ To test the hypothesis that a decrease in proteinuria would result in corresponding decreases in urinary KIM-1 levels as a marker of tubular damage, we investigated the effects of this stepwise decrease in proteinuria on urinary KIM-1 excretion. As a positive control for tubular injury, we also measured urinary excretion of *N*-acetyl- β -D-glucosaminidase (NAG).

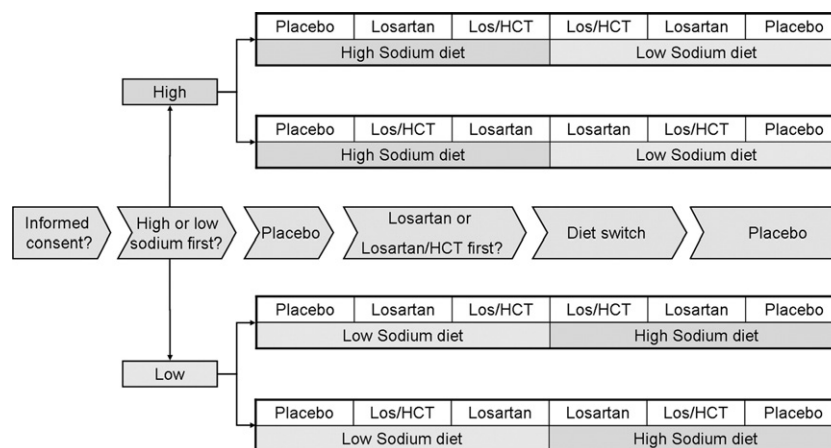


Figure 1. Flow diagram of the randomized controlled trial with crossover design. Selected patients entered the prospective, randomized, placebo-controlled, crossover study and were consecutively treated during 6 weeks with placebo, losartan (Los; 100 mg/d), and losartan plus hydrochlorothiazide (HCT; 100/25 mg/d) in random order. In addition, patients were randomized to start with a high (200 mmol sodium daily [\sim 4.8 g]) or a low-sodium diet (50 mmol sodium daily [\sim 1.2 g]) during 18 weeks (three 6-week periods). After 18 weeks, patients switched diet and the three 6-week periods were repeated. As can be deduced from the flow diagram, there are 4 randomization options to which patients were assigned.

METHODS

Patients and Protocol

This study is a post hoc analysis of a randomized, double-blind, placebo-controlled, crossover trial. The protocol was described in detail elsewhere.¹³ In brief, all included patients were aged 18 to 70 years and did not have diabetes. Patients had stable proteinuria with protein greater than 2 g and less than 10 g/d and stable kidney function (ie, creatinine clearance >30 mL/min and <6 mL/min/y decrease in the year preceding the study; creatinine clearance in mL/min may be converted to mL/s by multiplying by 0.01667). Every eligible patient entered the flow diagram of the trial shown in Fig 1. Additional antihypertensive drugs were allowed for blood pressure control, except for renin-angiotensin-aldosterone system (RAAS)-blocking agents or diuretics; these drugs were kept stable during the study.

The study design was prospective, randomized, placebo controlled, and crossover. Patients were treated for 6-week periods with placebo; losartan, 100 mg; and losartan plus HCT, 100/25 mg, in random order. In addition, patients were randomly assigned to either a high-sodium (HS) diet (200 mmol [\sim 4.8 g] of sodium daily) or an LS diet (50 mmol [\sim 1.2 g of sodium daily) during 18 weeks (three 6-week periods; sodium levels in mEq/L and mmol/L are equivalent). After 18 weeks, patients changed diet, and the three 6-week periods were repeated.

Assays and Measurements

Urinary protein was determined by using the pyrogallol red-molybdate method. Aliquots from the 24-hour urine collection were snap frozen in liquid nitrogen and stored (-20°C) until KIM-1 and NAG analysis. Urinary KIM-1 measurements were performed using microsphere-based Luminex xMAP technology (Luminex Corp, Austin, TX) with polyclonal antibodies raised against the human KIM-1 ectodomain. This technique is an adaptation of the previously described sandwich enzyme-linked immunosorbent assay.^{9,10,14} Urinary KIM-1 measured by using this enzyme-linked immunosorbent assay is known to mirror findings by means of Western blot analyses.¹⁵ For measurements, 30 μL of urine was analyzed in duplicate. The lower limit of detection for this assay is 4 pg/mL. The interassay and intra-assay variability was less than 10%. All measurements were performed by a blinded investigator (V.S.V.) who was unaware of patient characteristics. Reference values for urinary KIM-1 excretion were obtained from 24-hour urine measurements in 35 healthy volunteers. These were unmatched men, all white, with a mean age of 26 years (range, 20 to 67 years). Mean urinary KIM-1 excretion was 58 ± 8.0 (SE) ng/d, with a range of 0 to 156 ng/d and 95% confidence interval (CI) of 42 to 74. The cutoff value for normal (74 ng/d) was based on the upper limit of the 95% CI. Of note, urinary KIM-1 excretion was not related to age in healthy volunteers and nephrotic patients. KIM-1 excretion also was not related to sex in nephrotic patients. Urinary NAG was routinely assayed by using a modified enzyme assay according to Lockwood and Bosmann.¹⁶ In brief, 50 μL of untreated human urine was incubated in a final volume of 500 μL of reaction mix containing 2.4 mmol/L of substrate

(Sigma N9376; Sigma, Zwijndrecht, the Netherlands), 0.1 mol/L of citrate (pH 4.5), and 0.1% bovine serum albumin (Sigma A6152; Sigma) for 60 ± 2 minutes at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ in a Dubnoff metabolic shaker (Precision Scientific Inc, Chicago, IL). The reaction was terminated with 100 μL of 1.0 mol/L of sodium carbonate, and absorbance was read at 400 nm. A unit of enzyme activity is equivalent to 1 nmol of substrate hydrolyzed per hour. Reference values for urinary NAG excretion were obtained from 24-hour urine measurements in 20 healthy volunteers. These were all white individuals, 15 men and 5 women with a mean age of 58 years (range, 52 to 65 years). Mean urinary NAG excretion was 2.7 ± 0.5 U/d, with a range of 0.0 to 8.6 U/d and a 95% CI of 1.6 to 3.8. The cutoff value for normal (3.8 U/d) was based on the upper limit of the 95% CI. Serum and urinary creatinine were determined by using an automated multianalyzer (SMA-C; Technicon, Tarrytown, NY).

Blood pressure was measured under constant conditions at 1-minute intervals by using an automatic device (Dinamap; GE Medical Systems, Milwaukee, WI). After 15 minutes of measurements, the mean of the last 4 readings was used for further analysis. Mean arterial pressure was calculated as the sum of one-third systolic and two-thirds diastolic blood pressure.

Data Analysis

Results are expressed as mean \pm SE. Baseline data were obtained after 6-week placebo treatment with HS intake. Correlations between urinary KIM-1 levels and parameters such as proteinuria and blood pressure were analyzed using Pearson correlation coefficient. We used mixed-effects models for the analyses. These models include both fixed and random intercepts and fixed slopes to describe the longitudinal relationship (during the different modes of treatment) between a continuous outcome variable (KIM-1/NAG) and several other time-varying predictors (proteinuria, blood pressure, and creatinine clearance). For each of the 6 modes of treatments, a KIM-1 and NAG response for each patient was included in the models. The number of missing values per treatment was completely at random: 1 and 1 during HS-placebo for KIM-1 and NAG, 1 and 0 during LS-placebo, 0 and 1 during HS-losartan, 1 and 0 during LS-losartan, 0 and 0 during HS-losartan/HCT, and 3 and 3 during LS-losartan/HCT, respectively. Statistical significance was assumed at the 5% level of probability. We used SPSS, version 14.0 (SPSS Inc, Chicago, IL), for Windows (Microsoft Corp, Redmond, WA) for all analyses.

RESULTS

Patient Characteristics and Dietary Adherence

Thirty-four patients (25 men, 9 women; all white; mean age, 50 years; range, 23 to 68 years) were included. Baseline characteristics are listed in Table 1. Diagnoses were membranous glomerulopathy (7 patients), focal segmental glomerular sclerosis (8 patients), membranoproliferative glomerulonephritis (2 patients), minimal change disease with secondary glomerulosclerosis (2 pa-

Table 1. Baseline Characteristics

Men/women (n)	25/9
Race (white/African American/other)	34/0/0
Age (y)	50 (23–68)
Body weight (kg)	91 ± 3
Creatinine clearance (mL/min)	89 ± 5
Blood pressure (mm Hg)	
Systolic	143 ± 4
Diastolic	86 ± 2
Proteinuria (g/d)	3.8 ± 0.4
Kidney injury molecule 1 (ng/d)	1,706 ± 498
<i>N</i> -Acetyl- β -D-glucosaminidase (U/d)	12.8 ± 1.2
Diagnoses	
Membranous glomerulopathy	7
Focal segmental glomerular sclerosis	8
Membranoproliferative glomerulonephritis	2
Minimal change disease with secondary glomerulosclerosis	2
Hypertensive nephropathy	5
Immunoglobulin A nephropathy	5
Alport syndrome	1
Not otherwise specified, nonconclusive diagnosis	4

Note: Data expressed as mean \pm SE or number (range) unless stated otherwise. Creatinine clearance in mL/min may be converted to mL/s by multiplying by 0.01667.

tients), hypertensive nephropathy (5 patients), immunoglobulin A nephropathy (5 patients), Alport syndrome (1 patient), and nonconclusive diagnosis (4 patients). One patient (diagnosis of focal segmental glomerular sclerosis) could not fulfill the complete protocol (because of psycho-

logical distress unrelated to the study medication) and was excluded from analysis. Dietary adherence was adequate, indicated by mean urinary sodium excretion of 196 ± 9 mEq/d during the HS periods and 92 ± 8 mEq/d ($P < 0.001$) during the LS periods.

Effects of Treatment on Urinary KIM-1 and NAG Levels, Proteinuria, Blood Pressure, and Kidney Function

Urinary KIM-1 levels were greatest during the baseline period of placebo on HS intake and considerably greater than the reference values obtained in healthy volunteers. The interventions induced a stepwise decrease in proteinuria (Fig 2, left panel) and blood pressure (Fig 2, left lower panel) that was accompanied by a parallel stepwise decrease in urinary KIM-1 levels (Fig 2, middle panel; $P = 0.009$ for trend). The decrease in urinary KIM-1 levels was statistically significant during all treatment periods, with the single exception of the losartan/HS period, for which significance was borderline ($P = 0.09$). However, the lowest mean value for KIM-1, obtained during losartan/LS plus HCT (743 ± 170 ng/d), was still 10 times greater than the upper limit of normal (Fig 2, middle panel). Urinary NAG excretion also was considerably greater than the reference value obtained in healthy volunteers. Losartan monotherapy or LS diet only did not

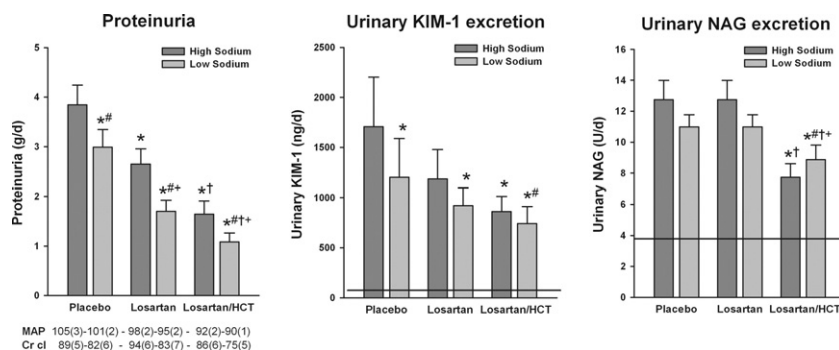


Figure 2. Urinary kidney injury molecule 1 (KIM-1) and *N*-acetyl- β -D-glucosaminidase (NAG) excretion during the different treatment modalities. Left dark gray bars, high-sodium diet; right light gray bars, low-sodium diet. (Left lower panel) Blood pressure (mm Hg) and creatinine clearance (Cr Cl; mL/min/1.73 m₂) during the different treatment periods presented below the bars that represent proteinuria levels during that period. * $P < 0.05$ versus placebo high sodium (HS; baseline); # $P < 0.05$ versus same treatment on HS (effect of low sodium). The reference value for urinary KIM-1 excretion is based on the upper limit of the 95% confidence interval (CI) of KIM-1 measurements in 24-hour urine collections from healthy volunteers ($n = 35$, see Methods for characteristics) and indicated by the black line (74 ng/d). The reference value for urinary NAG excretion is based on the upper limit of the 95% CI of NAG measurements in 24-hour urine collections from healthy volunteers ($n = 20$; see Methods for characteristics) and indicated by the black line (3.8 U/d). Creatinine clearance in mL/min may be converted to mL/s by multiplying by 0.01667. Abbreviations: HCT, hydrochlorothiazide; MAP, mean arterial pressure.

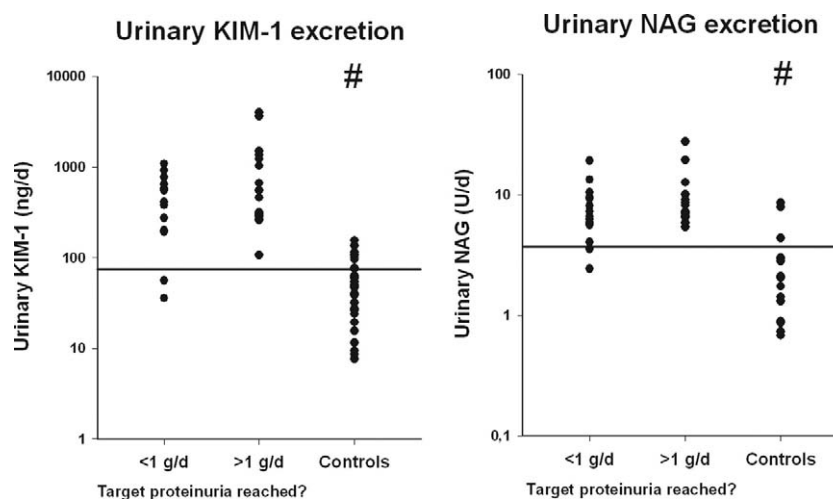


Figure 3. Urinary kidney injury molecule 1 (KIM-1) and *N*-acetyl- β -D-glucosaminidase (NAG) excretion is lower in patients who reach target proteinuria. Patients were divided according to whether they reached target proteinuria of protein less than 1 g/d (16 of 33) or not (17 of 33). Also, individual urinary control values for KIM-1 and NAG are plotted ($n = 35$ and $n = 20$, respectively). Black horizontal line, reference values for KIM-1 and NAG. To clearly show KIM-1 and NAG values in the lower range, the y-axis on which urinary KIM-1 and NAG are expressed is shown on a logarithmic scale. After maximal antiproteinuric treatment, urinary KIM-1 and NAG excretion was normalized in only 2 of 16 patients who reached target proteinuria. # $P < 0.05$ versus both nephrotic patients who reach target proteinuria and nephrotic patients who do not reach target proteinuria.

exert significant effects on urinary NAG excretion. However, during the combination of losartan with HCT, urinary NAG excretion significantly decreased during either diet (Fig 2, right panel).

Whereas target proteinuria with protein less than 1 g/d was reached in 16 of 33 patients, urinary KIM-1 levels normalized in only 2 of these patients (Fig 3), and urinary KIM-1 levels were still significantly increased versus control values. The same applied to urinary NAG values. On mixed-model analyses, KIM-1 and NAG excretion were not influenced by the order of treatment periods or number of subsequent active treatment periods.

Creatinine clearance was significantly lower during all sodium-restricted periods compared with the corresponding HS periods. Losartan did not affect creatinine clearance during either diet (Fig 2, left lower panel).

Urinary KIM-1 and NAG Significantly Correlated With Decrease in Proteinuria, But Not With Blood Pressure or Kidney Function

To analyze whether decreases in KIM-1 and NAG levels might be related to specific effects of intervention on the kidney, we analyzed the association between urinary levels of KIM-1 and

effects of intervention on proteinuria, blood pressure, and creatinine clearance. Univariate analyses of the separate treatment periods show that urinary KIM-1 and NAG levels correlated significantly with proteinuria during all treatment periods, except during losartan on an LS diet (data not shown). Conversely, urinary KIM-1 and NAG levels did not correlate with blood pressure or creatinine clearance during any of the treatment periods (data not shown). Associations of mean KIM-1 and NAG levels per treatment period with the corresponding proteinuria values are shown in Fig 4. In Figs 2 and 4, urinary KIM-1 excretion appears more closely related to proteinuria than urinary NAG excretion. To test whether the proteinuria-associated decreases in KIM-1 and NAG levels were related to treatment modality, we performed partial correlation analysis for all treatment periods combined, controlling for mode of intervention. In this analysis, urinary KIM-1 and NAG levels were significantly related to proteinuria decrease ($R = 0.534$; $P < 0.001$ and $R = 0.652$; $P < 0.001$, respectively). In contrast to proteinuria, partial correlation coefficients between urinary KIM-1 level and blood pressure or kidney function (measured as creatinine clearance) were not significant. Finally, we performed a mixed-model analysis with urinary

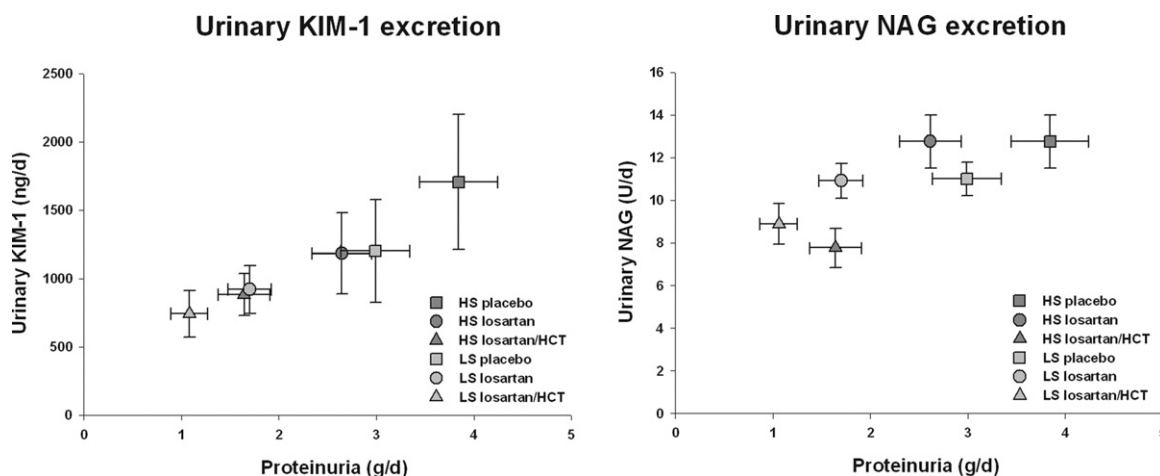


Figure 4. Antiproteinuric treatment decreases urinary kidney injury molecule 1 (KIM-1) and *N*-acetyl- β -D-glucosaminidase (NAG) excretion. In these between-treatment period graphs, urinary KIM-1 excretion appears more closely related to proteinuria than urinary NAG excretion. Abbreviations: HCT, hydrochlorothiazide; HS, high sodium; LS, low sodium.

KIM-1 and NAG levels as dependent variables. Mode of treatment was a significant predictor of urinary KIM-1 (estimate, -165 ng/d; $P < 0.001$) and urinary NAG excretion (-0.88 U/d; $P < 0.001$). However, when proteinuria was included in the models, mode of treatment was not a significant predictor of KIM-1 (estimate, 35 ng/d; $P = 0.7$) and NAG (0.29 U/d; $P = 0.1$). The fixed-effects parameter estimates show that proteinuria was related to the initial status of KIM-1 excretion (estimate, 289 ng/d; $P < 0.01$) and rate of change (-63 ng/d; $P < 0.05$). Conversely, proteinuria was related to only the initial status of NAG (1.9 U/d; $P < 0.001$) and not the rate of change (0.11 U/d; $P = 0.2$). Mean arterial pressure was not related to initial status or rate of change in urinary KIM-1 and NAG excretion (-4.4 ng/d; $P = 0.8$ for initial status; 3.5 ng/d; $P = 0.4$ for rate of change for KIM-1; -0.03 U/d; $P = 0.6$ and 0.005 U/d; $P = 0.7$ for NAG). Similarly, creatinine clearance was not related to initial status or rate of change in urinary KIM-1 and NAG excretion (-12.7 ng/d; $P = 0.1$ for initial status; 3.6 ng/d; $P = 0.2$ for rate of change for KIM-1; 0.03 U/d; $P = 0.1$ and -0.001 U/d; $P = 0.8$ for NAG). These results are consistent with the hypothesis of amelioration of proteinuria-induced tubular damage.

DISCUSSION

In proteinuric patients without diabetes with well-preserved and stable kidney function, uri-

nary KIM-1 and NAG excretion were markedly increased, indicating tubular damage. Antiproteinuric treatment decreased urinary KIM-1 excretion in these patients, which was quantitatively related to the efficacy of proteinuria reduction, but not to blood pressure. Because the decrease in urinary KIM-1 excretion also occurred during the placebo/LS period, it appears to be caused by the lower proteinuria as such, rather than RAAS blockade. Because KIM-1 expression is associated with the early tubular responses to damage, the decrease in urinary KIM-1 levels by antiproteinuric treatment suggests that it reflects amelioration of proteinuria-associated tubulointerstitial pathways of damage.

We have no conclusive proof that the decrease in urinary KIM-1 levels reflects a decrease in tubular expression of KIM-1. However, in experimental kidney disease, we recently showed that tubular expression of KIM-1 decreased in relation to the decrease in proteinuria by means of RAAS blockade in Adriamycin nephropathy¹⁷ and homozygous Ren2 rats.¹⁸ Furthermore, there was a very high correlation between Kim-1 messenger RNA levels and urinary Kim-1 excretion in rats exposed to varying periods of ischemia.⁹ Those studies support the assumption that the decrease in KIM-1 levels observed here reflects a decrease in tubular expression of KIM-1, but for obvious reasons, no kidney biopsy data are available to provide conclusive proof.

Because proteinuria is the main risk factor for decreased kidney function, a decrease in proteinuria to protein less than 1 g/d is advocated for clinical renoprotection.^{6,7} The decrease in proteinuria was consistently associated with better long-term kidney outcome. However, despite maximal antiproteinuric treatment by using RAAS blockade combined with optimization strategies such as dual blockade¹⁹ and intervention in volume status by means of an LS diet and diuretics, in many patients, the decrease in kidney function was just slowed, but not halted or reversed.^{20,21} Thus, further improvement in current renoprotective intervention strategies is required. In our study, proteinuria reached the target level of protein less than 1 g/d in 16 of 33 patients. However, even when proteinuria decreased to protein less than 1 g/d, urinary KIM-1 and NAG levels did not normalize in 14 of 16 patients, suggesting ongoing tubular damage. If so, this could implicate that target proteinuria with less than 1 g/d of protein is still too liberal to confer optimal renoprotection. It could be argued that our treatment periods lasted only 6 weeks and reversal of the proteinuria-induced tubulointerstitial inflammatory state would require more time. Therefore, we also tested whether urinary KIM-1 and NAG levels were lower at the end of 2 subsequent active treatment periods. This was not the case, suggesting that longer duration of treatment would not result in a further decrease in urinary KIM-1 levels. Long-term studies are needed to determine the prognostic impact of persistent increases in urinary KIM-1 and NAG levels in patients with proteinuria with less than 1 g/d of protein.

The prognostic impact of KIM-1 level relative to that of proteinuria needs substantiation from prospective long-term data and cannot be derived from our present data. However, it should be mentioned that renal interstitial damage can dissociate from proteinuria, and thus might have an independent prognostic impact. It is well established that renal tubulointerstitial damage is a consistent predictor of renal prognosis in studies documenting renal morphological data^{22,23} and those addressing the prognostic impact of urinary markers of tubular dysfunction or damage. Greater excretion of low-molecular-weight (“tubular”) proteins in urine is associated with faster progression of CKD.²⁴ The combination of

nonselective proteinuria and high fractional excretion of α_1 -microglobulin predicts the progression of kidney disease in proteinuric patients.²⁵ In patients with membranous nephropathy, high urinary NAG excretion predicts progression of CKD even better than proteinuria.²⁶ In experimental studies, pronounced progression of renal interstitial damage has been documented during RAAS blockade despite an effective decrease in proteinuria.²⁷ Therefore, therapy response to proteinuria and renal tubulointerstitial damage can dissociate, suggesting that biomarkers for tubulointerstitial damage could be valuable as prognostic markers, either as such or more likely in combination with proteinuria. In acute kidney injury, urinary KIM-1 levels have better prognostic value than such conventionally used severity markers as urine output and serum creatinine values.¹⁴ Furthermore, in kidney transplant recipients, greater urinary KIM-1 excretion predicts renal outcome, showing that the prognostic impact of KIM-1 is not limited to acute kidney disease.¹² Short-term decreases in proteinuria predict a slower decrease in glomerular filtration rate in patients with nondiabetic and diabetic nephropathy.⁶ The prognostic impact of the observed short-term effects of antiproteinuric intervention on KIM-1 excretion needs to be investigated further in long-term studies. Based on our present study together with prior studies, urinary KIM-1 excretion, a biomarker for tubular damage, seems a promising tool to monitor therapy response and target intervention. To establish the prognostic impact of KIM-1 relative to proteinuria, future long-term studies should investigate whether glomerular (proteinuria) and interstitial markers (KIM-1) have independent prognostic impact and thus could provide independent treatment targets. If so, it might be worthwhile to test whether targeting treatment on KIM-1, in addition to proteinuria, can improve outcomes in patients with progressive kidney function loss.

Urinary KIM-1 level is a sensitive noninvasive indicator for tubular damage. In a comparison study of the sensitivity of different kidney damage markers in rats, using increasing doses of nephrotoxins, KIM-1 was found to be a more sensitive marker of kidney injury than NAG.²⁸ Our present finding that NAG levels decreased only during the treatment periods with the largest effects on proteinuria can be considered consis-

tent with this. A problem with NAG as a biomarker is that its activity can be inhibited by some nephrotoxicants.^{29,30}

Our study is not designed to elucidate the mechanism of KIM-1 excretion in urine, but the most probable explanation is shedding of the KIM-1 ectodomain in response to tubular epithelial cell handling of proteins. Because KIM-1 is expressed at the luminal side of proximal tubular cells, it is not likely that the decreased urinary excretion of KIM-1 during antiproteinuric treatment is directly linked to alteration in glomerular filtration rate, also supported by the absence of a relation between KIM-1 level and creatinine clearance. The decrease in urinary KIM-1 levels may reflect amelioration of proteinuria-associated tubulointerstitial damage. This is supported by the decrease in urinary excretion of NAG, an established marker of tubulointerstitial damage, during the treatment periods with the most effective decrease in proteinuria, ie, combined losartan and HCT. Earlier studies support the beneficial effects of RAAS blockade on tubulointerstitial damage. Recently, it was shown that the addition of spironolactone to dual RAAS blockade decreased NAG excretion in an open controlled crossover study of 18 patients with nondiabetic proteinuria.³¹ In 7 pediatric patients with immunoglobulin A nephropathy, NAG levels tended to decrease with angiotensin-converting enzyme inhibitor therapy and the combination with angiotensin II type 1 receptor blockade.³² Because studies of the impact of intervention on tubular markers are largely open labeled, retrospective, and nonrandomized, this issue deserves better consideration in prospective, placebo-controlled, and randomized studies.

However, alternative explanations for the decrease in urinary KIM-1 levels also should be considered. KIM-1 is a type I transmembrane glycoprotein with an ectodomain containing an immunoglobulin-like domain and a mucin domain. The cell-surface (mature) form of KIM-1 is 100 kd, and the shedded soluble KIM-1 protein is about 90 kd. There are 2 human homologs of KIM-1: KIM-1a and KIM-1b.³³ These homologs are identical except for the C-terminal portion of their cytoplasmic domain. Analysis of their genomic structure and complementary DNA products indicates that they are splice variants and shows that KIM-1a is the major form in the liver and KIM-1b is predominant in the kidney.³³

Theoretically, KIM-1 might be shed from the liver into the circulation and thus be a source of urinary KIM-1 in proteinuric patients. Studies of the possible presence of KIM-1 in the circulation are currently under way. Because we have no data about the possible presence of KIM-1 in the circulation or the selectivity index of proteinuria in our patients, we cannot fully exclude the possibility that the urinary KIM-1 in our study is partly from nontubular origin. Also, a role for urinary matrix metalloproteinases (MMPs) should be considered. In normal kidney tissue, KIM-1 is undetectable. However, it is abundantly expressed in proximal tubular cells in various human kidney diseases.¹⁰ Shedding of this ectodomain is confirmed in cultured human proximal tubular cells.³³ In these cells, the cleavage and shedding could be blocked by hydroxamic acid-based zinc MMP inhibitors, suggesting this is an active process mediated by a metalloproteinase.³³ Urinary excretion of MMP is increased in patients with CKD, antineutrophil cytoplasm autoantibody-associated vasculitis, and type 1 diabetes in the presence of markers of kidney damage.³⁴⁻³⁶ Thus, the possible presence of MMPs in the tubular lumen could have affected the processing of KIM-1 in the urine.

In our study, KIM-1 did not correlate with severity of kidney function impairment, which is at variance with a prior study from our center.¹⁰ However, in the present study, kidney function was relatively well preserved and the range of kidney function was relatively small. Van Timmeren et al¹⁰ studied patients with a wider range of kidney disorders with a wider range of kidney function impairment. This made the likelihood to detect an association between urinary KIM-1 level and kidney function larger than in the present study.

In conclusion, in patients without diabetes with proteinuria with well-preserved and stable kidney function, urinary KIM-1 and NAG levels are markedly increased. Urinary KIM-1 level is decreased by antiproteinuric treatment irrespective of the mode of intervention. NAG level also decreased, but only during the periods with the strongest proteinuria decrease. The decrease in KIM-1 levels suggests that proteinuria-induced tubular damage is ameliorated by antiproteinuric intervention. However, even when proteinuria was decreased to less than 1 g/d of protein, urinary KIM-1 and NAG levels did not normalize in all except 2 patients, suggesting ongo-

ing tubular damage. Long-term studies are warranted to evaluate whether targeting treatment on KIM-1 as a parameter of tubular damage can improve outcome in patients with proteinuria-associated CKD.

ACKNOWLEDGEMENTS

We thank Corrie Nieuwenhout, BSc, for skillful assistance at the outpatient clinic; Nynke Halbesma, MSc, for assistance with statistical analyses; Jacco Zwaagstra, BSc, for technical assistance; and Mienke Rook, MD, for critically reviewing the manuscript.

Support: This study was supported by Merck Sharp & Dohme (grant MSGP NETH-15-01). The work in Dr Bonventre's laboratory was supported by National Institutes of Health grants DK39773, DK072381, and DK074099. Dr Vaidya was supported by a scientist development grant (0535492T) from the American Heart Association and grant K99 ES16723 from the National Institute of Environmental Health Sciences. Dr Damman is supported by the Netherlands Heart Foundation (grant 2006B157).

Financial Disclosure: None.

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