Increased Expression of Mineralocorticoid Effector Mechanisms in Kidney Biopsies of Patients With Heavy Proteinuria

Marcus Quinkler, MD; Daniel Zehnder, MD, PhD; Kevin S. Eardley, MRCP; Julia Lepenies, MD; Alexander J. Howie, MD, FRCPath; Susan V. Hughes, HNC; Paul Cockwell, PhD; Martin Hewison, PhD; Paul M. Stewart, MD, FRCP

Background—Aldosterone has emerged as a deleterious hormone in the heart, with mineralocorticoid receptor (MR) blockade reducing mortality in patients with severe heart failure. There is also experimental evidence that aldosterone contributes to the development of nephrosclerosis and renal fibrosis in rodent models, but little is known of its role in clinical renal disease.

Methods and Results—We quantified MR, serum- and glucocorticoid-regulated kinase 1 (sgk1), and mRNA expression of inflammatory mediators such as macrophage chemoattractant protein-1 (MCP-1), transforming growth factor-β1, and interleukin-6 in 95 human kidney biopsies in patients with renal failure and mild to marked proteinuria of diverse etiologic origins. We measured renal function, serum aldosterone, urinary MCP-1 protein excretion, and the amount of chronic renal damage. Macrophage invasion was quantified by CD68 and vascularization by CD34 immunostaining. Serum aldosterone correlated negatively with creatinine clearance (P<0.01) and positively with renal scarring (P<0.05) but did not correlate with MR mRNA expression or proteinuria. Patients with heavy albuminuria (>2 g/24 h; n=15) had the most renal scarring and the lowest endothelial CD34 staining. This group showed a significant 5-fold increase in MR, a 2.5-fold increase in sgk1 expression and a significant increase in inflammatory mediators (7-fold increase in MCP-1, 3-fold increase in transforming growth factor-β1, and 2-fold increase in interleukin-6 mRNA). Urinary MCP-1 protein excretion and renal macrophage invasion were significantly increased in patients with heavy albuminuria.

Conclusions—These studies support animal data linking aldosterone/MR activation to renal inflammation and proteinuria. Further studies are urgently required to assess the potential beneficial effects of MR antagonism in patients with renal disease. (Circulation. 2005;112:1435-1443.)

Key Words: blood pressure ■ hormones ■ hypertension, renal ■ kidney ■ receptors

Inhibitors of ACE and angiotensin receptor blockers have shown efficacy in slowing the progression of both experimental and clinical renal disease.1-5 Angiotensin II has been regarded as the major mediator of injurious actions of the renin-angiotensin-aldosterone system in the kidney by elevating glomerular pressure, mediating vasoconstriction, and promoting fibroproliferative effects. Recently, aldosterone (associated with salt) has also been proposed as a deleterious mediator of the renin-angiotensin-aldosterone system in the heart and kidney.6,7 In 1999, the RALES study showed that mineralocorticoid receptor (MR) blockade by spironolactone reduced morbidity in patients with severe heart failure.7 Four years later, the beneficial effect of MR blockade with the newer antagonist eplerenone was shown in patients with left ventricular dysfunction after myocardial infarction.8 There is considerable experimental evidence that aldosterone also contributes to the development of renal fibrosis in several models. First, aldosterone produces arterial hypertension, proteinuria, and glomerulosclerosis despite ACE inhibitors and angiotensin receptor blockers in the rat remnant kidney model.4 Second, stroke-prone spontaneously hypertensive rats have higher renal concentrations of MR than normotensive Wistar-Kyoto rats,9 and spironolactone reduced proteinuria10 and prevented malignant nephrosclerosis.11 In salt-loaded rats (eg, spontaneously hypertensive rats, stroke-prone spontaneously hypertensive rats), plasma aldosterone is presumably suppressed. The efficacy of spironolactone in this setting raises the possibility of local aldosterone production and its autocrine/paracrine properties.12 Third, in the NO-citrulline nitro-l-arginine methyl ester rat model, MR blockade was
nephroprotective without altering blood pressure.\textsuperscript{13} Finally, the inflammatory effects of aldosterone in the rat include increased renal expression of proinflammatory cytokines such as monocyte chemoattractant protein-1 (MCP-1), with interstitial macrophage infiltration and injury.\textsuperscript{14} Tubular epithelial cells are considered a central cell type in renal inflammation because they are able to produce a large variety of cytokines\textsuperscript{15} such as interleukin (IL)-6, chemokines such as IL-8 and MCP-1, and profibrotic factors, including transforming growth factor-\beta1 (TGF-\beta-1). Tubular epithelial cell activation is triggered by hypoxia or protein overload resulting from enhanced glomerular permeability.\textsuperscript{16,17} Cross-talk between tubular epithelial cells and infiltrating leukocytes regulates inflammatory mediators and may lead to further attraction of inflammatory cells.

Patients with stable chronic renal insufficiency show higher plasma aldosterone levels than healthy individuals.\textsuperscript{18,19} Most patients with aldosteronism have proteinuria, present often with increased urinary calcium and magnesium excretion, and show hypomagnesemia.\textsuperscript{20} There are a few small, preliminary, encouraging clinical trials using MR blockers to attenuate chronic renal injury.\textsuperscript{21–23}

To the best of our knowledge, nothing has been published about renal MR expression and MR effector mechanisms in human kidney disease. Therefore, we have investigated the expression of MR and its target gene serum- and glucocorticoid-regulated kinase 1 (sgk1) and their correlation with inflammatory markers and serum aldosterone concentrations in 95 patients with renal disease undergoing diagnostic biopsy. The aim of this study was to provide further evidence that aldosterone influences renal inflammation and the expression of proinflammatory cytokines via the MR in humans.

**Methods**

**Subjects and Clinical Data**

All 95 patients (53 men, 42 women), recruited from the Department of Nephrology at Queen Elizabeth Hospital (Birmingham, UK), underwent kidney biopsies to investigate proteinuria and/or hematuria and/or renal impairment. Patients with acute renal failure were not included; those taking spironolactone also were excluded. The study was approved by the local research ethics committee, and all patients gave written informed consent before study inclusion. Renal biopsy material was divided, and part was rapidly frozen at −80°C. The rest was fixed in formaline-saline and glutaraldehyde for routine pathological study. Blood pressure was measured 3 times before the biopsy, and the average was calculated. Twenty-four-hour urine collection was performed before renal biopsy. Urinary MCP-1 protein was quantified with an ELISA kit (Duoset, R&D Systems).

Creatinine clearance (CCR; mL/min) was calculated by the Cockroft-Gault formula\textsuperscript{24}: 

$$\text{CCR} = \frac{140 - \text{age (years)}}{\times \text{weight (kg)}} \times \text{mg creatinine concentration (mmol/L)}$$

Urea excretion was measured by radioimmunoassay (Coat-A-Count \textsuperscript{125}I-Aldosterone RIA Kit, Diagnostic Products Corp.). The main diagnoses are shown in Table 1 and the relevant clinical data in Table 2.

Forty-four patients had an underlying diagnosis of hypertension and received antihypertensive therapy: 10 with single therapy, 22 with double therapy, 6 with 3 drugs, and 6 with 4 drugs. Eighteen received furosemide, 10 took ACE inhibitors, and 5 received thiazides given either as single therapy or in combination with each other or with calcium antagonists or \beta-blockers. Eleven patients were taking regular prednisolone in doses of ≥5 mg/d. Four patients without hypertension were taking ACE inhibitors for proteinuria. The various factors were analyzed for their relation to 4 variables: renal function, blood pressure, diagnosis, and proteinuria. For analysis with regard to proteinuria, patients were divided into 4 groups: no albuminuria (<30 mg/24 h; n = 30), microalbuminuria (30 to 300 mg/24 h; n = 26), moderate albuminuria (300 mg to 2 g/24 h; n = 17), and heavy albuminuria (>2 g/24 h; n = 14). Four patients with heavy albuminuria had nephrotic syndrome.

**RNA Extraction and RT**

Biopsies were homogenized on ice with a metal homogenizer treated with RNase Zap (Ambion). Homogenates were centrifuged at 12,000g for 10 minutes at 4°C. Total RNA was extracted from supernatants with the Tri Reagent extraction method (Sigma UK). RNA integrity was assessed by electrophoresis on 1% agarose gels; the quantity determined spectrophotometrically at OD\textsubscript{260} 1 μg of total RNA was initially denatured by heating to 70°C for 5 minutes. Then, 30 U avian myeloblastosis virus, 200 ng random primers, 20 U ribonuclease inhibitor, and 40 nmol deoxy-NTPs with 5× reaction buffer were added to the RNA, and the RT reaction was carried out at 37°C for 1 hour. The reaction was terminated by heating the cDNA to 95°C for 5 minutes.

**Quantitative RT-PCR**

MR, sgk1, MCP-1, TGF-\beta-1, and IL-6 mRNA expression levels were analyzed with an ABI Prism 7700 sequence detection system.

**TABLE 1. Pathological Diagnoses on Renal Biopsies**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thin glomerular basement membrane disease</td>
<td>23</td>
</tr>
<tr>
<td>Chronic ischemic renal damage</td>
<td>22</td>
</tr>
<tr>
<td>IgA nephropathy</td>
<td>18</td>
</tr>
<tr>
<td>Focal segmental sclerosing glomerulonephritis</td>
<td>8</td>
</tr>
<tr>
<td>Diabetic glomerulopathy</td>
<td>5</td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td>3</td>
</tr>
<tr>
<td>Mild IgM mesangial glomerulonephritis</td>
<td>3</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>3</td>
</tr>
<tr>
<td>Nodular light-chain glomerulopathy with myeloma</td>
<td>3</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>2</td>
</tr>
<tr>
<td>Minimal change nephropathy</td>
<td>2</td>
</tr>
<tr>
<td>Membranous nephropathy with renal vasculitis</td>
<td>1</td>
</tr>
<tr>
<td>Interstitial nephritis</td>
<td>1</td>
</tr>
<tr>
<td>Metastatic carcinoma in kidney</td>
<td>1</td>
</tr>
</tbody>
</table>

n=95.

**TABLE 2. Clinical Data of Patients Who Underwent Kidney Biopsies**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>49.3</td>
<td>18–83</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.8</td>
<td>16.6–44.5</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>134</td>
<td>90–171</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>74</td>
<td>45–96</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mm Hg</td>
<td>94</td>
<td>60–117</td>
</tr>
<tr>
<td>Serum albumin, g/L</td>
<td>38.3</td>
<td>10–50</td>
</tr>
<tr>
<td>Urea, mmol/L</td>
<td>10</td>
<td>2.2–35.6</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>12.6</td>
<td>6.1–17.7</td>
</tr>
<tr>
<td>Platelets, ×10³/mL</td>
<td>258</td>
<td>121–614</td>
</tr>
<tr>
<td>Serum creatinine, mmol/L</td>
<td>177</td>
<td>67–1110</td>
</tr>
<tr>
<td>Creatinine clearance, mL/min</td>
<td>68.6</td>
<td>7.4–150.1</td>
</tr>
<tr>
<td>Urinary albumin excretion, g/24 h</td>
<td>1.25</td>
<td>0–18</td>
</tr>
</tbody>
</table>

n=95.
Renal MR and sgk1 Expression in Proteinuria

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(Perkin-Elmer Applied Biosystems) as previously described.28 Real-time RT-PCR was performed in 25-µL volumes on 96-well plates in reaction buffer containing TaqMan Universal PCR Master Mix (Applied Biosystems) and 25 ng cDNA template. All reactions were singleplexed with the housekeeping gene (18S ribosomal RNA gene) provided as preoptimized control probe and primers (Applied Biosystems), enabling data to be expressed in relation to an internal reference to allow for differences in RT efficiency. Measurements were carried out at least 3 times for each sample. All target gene probes were labeled with the fluorescent probe dye TAMRA, and the housekeeping gene was labeled with the fluorescent reporter dye VIC. Reactions were as follows: 50°C for 2 minutes, 95°C for 10 minutes, and then 44 cycles of 95°C for 15 seconds and 60°C for 1 minute. Oligonucleotide primers and a Taqman probe for MR were as follows: forward, CCAAAATGCTCTTCAGTTTGCIT; reverse, TTAGGCGCCTCCTTGGGAA; and probe, TTGAGAATA-CACCATCATGAAAGTTTGCTGACTAG. The primers and probe used for sgk1, MCP-1, TGFβ-1, and IL-6 real-time PCR were commercially available Assays on Demand (Applied Biosystems).

According to the manufacturer’s guidelines, data were expressed as ct values (the cycle number at which logarithmic PCR plots crossed a calculated threshold line) and used to determine Δct values (Δct equals ct of the target gene minus ct of the housekeeping gene). High Δct values represent low levels of expression. Fold changes in expression were calculated according to the transformation: fold increase = 2^Δct. Statistical Analysis

Data are expressed as mean±SD unless otherwise stated. Statistical analysis on real-time PCR data was performed on mean Δct values (not on fold changes) to exclude potential bias owing to averaging data that had been transformed through the equation 2^Δct. Statistical analysis of comparisons between groups was undertaken with paired and unpaired t tests or ANOVA when appropriate; otherwise, the Mann-Whitney rank-sum test was used. For correlations, a stepwise multiple regression analysis was performed with SPSS (SPSS Inc) and Pearson’s correlations.

Results

Renal Function

Using a cutoff of 60 mL/min for creatinine clearance,28 we found that 58 patients had good or slightly impaired renal function, and 37 had moderately or severely impaired renal function. Patients with moderately or severely impaired renal function had higher blood pressure than the other patients, both systolic (142.7±15.6 versus 128.9±17.4 mm Hg; P<0.001) and diastolic (77±±11.0 versus 72.5±10.7 mm Hg; P<0.05). Patients with moderately or severely impaired renal function showed no significant changes in MR, sgk1, TGFβ-1, or IL-6 mRNA expression compared with patients with good or slightly impaired renal function. MCP-1 mRNA expression showed a significant negative correlation with creatinine clearance (Figure 1A). Patients with moderately or severely impaired renal function showed a higher urinary MCP-1 protein excretion (353±343 versus 180±231 pg/mg creatinine; P<0.05), a higher index of chronic damage (46.2±31.2% versus 7.3±11.9%; P<0.001), less staining for endothelial marker CD34 (6.4±2.4% versus 9.9±2.2%; P<0.001), and more staining for macrophage antigen CD68 (2.7±1.8% versus 1.2±0.5%; P<0.001). There was a significantly negative correlation between serum aldosterone concentration and renal function (Figure 1B). Aldosterone concentration did not correlate with serum potassium, calcium, or magnesium levels or MR mRNA expression but had a significant positive correlation with renal scarring (Figure 1C).

Blood Pressure

Forty-four patients on treatment for hypertension had significant higher blood pressure than the other patients (systolic, 142.7±15.5 versus 127.0±16.8 mm Hg; P<0.001; diastolic, 78.5±9.5 versus 70.7±11.1 mm Hg; P<0.001). The hypertensive patients had a lower creatinine clearance (47.7±27.8 versus 85.4±35.1 mL/min; P<0.001), a higher index of chronic damage (scarring index, 34.4±29.9% versus 10.5±21.3%; P<0.001), less CD34 staining (7.1±2.6% versus 9.9±2.3%; P<0.001), more CD68 staining (2.1±1.2% versus 1.4±1.3%; P<0.05), and higher serum aldosterone concentration (472±284 versus 324±230 pmol/L; P<0.05). There were no differences in MR, sgk1, MCP-1, TGFβ-1, or IL-6 mRNA expression between the 2 groups. No correlation was found between systolic or diastolic blood pressure and MR, sgk1, TGFβ-1, or IL-6 mRNA expression, but there was a significant positive correlation between systolic blood pressure and MCP-1 mRNA expression (r=0.359, P<0.005) and between systolic blood pressure and renal scarring (r=0.314, P<0.01).

Measurement of Chronic Damage in Biopsy Specimens

An interactive image analysis system was used to outline and measure the extent of glomerular and interstitial scarring, expressed as percentage of cortical cross-sectional area, in renal biopsy stained with periodic acid–methenamine silver. This measure, the index of chronic damage, was shown to be a strong predictor of renal outcome.26 Immunohistochemistry on Biopsy Specimens

Detection of tissue macrophages, endothelial cells, and MR was performed with immunohistochemistry. Briefly, paraffin-embedded sections were processed in 0.01 mol/L sodium citrate buffer (pH 6.0) kept at 95°C in a microwave for 30 minutes. Slides were then incubated with methanol-hydrogen peroxide (1:10), followed by sequential treatment with 0.1% avidin, 0.01% biotin (X0590 Dako Ltd Ely, UK) and hydrogen peroxide. Slides used for qualitative analysis for macrophage antigen CD68 (2.7±1.8% versus 9.9±2.2%; P<0.001), and 10% rabbit serum. Three-stage indirect immunohistochemistry was performed with primary mouse monoclonal antibodies directed against the pan-macrophage antigen CD68 (5 µg/mL; clone PG-M1; Dako Ltd Ely UK) or the endothelial marker CD34 (1 µg/mL; clone QBEnd 10; Dako Ltd Ely, UK), each for 1 hour, or a mouse antibody (1:20) directed against the MR (generous gift from C. Gomez-Sanchez) for 16 hours. Biotinylated secondary rabbit anti-mouse antibody (50 µg/mL; Dako Ltd Ely, UK) was applied for 1 hour and by horseradish peroxidase–conjugated streptavidin ABC complex (Dako Ltd Ely, UK) for 20 minutes. Binding was visualized with 3′,3′-diaminobenzidine (Vector Laboratories Ltd) and hydrogen peroxide. Slides used for qualitative analysis of CD68 or CD34 were then counterstained with Mayer’s hematoxylin; those used for quantitative analysis were not. An isotype control (IgG1; Dako Ltd) consistently resulted in no positive staining.

An interactive image analysis system was used to carry out blinded quantitative analysis of the extent of CD68 and CD34 immunostaining in biopsy specimens, expressed as percentage of cortical cross-sectional area. This technique was found to be reliable in the analysis of human and animal renal sections.27 Images at magnification ×200 were captured and converted to a 2-color scale image with an Aequitas IDA software system (Dynamic Data Links). For each patient, the mean measurement of 5 randomly selected nonconfluent microscopic fields was determined. Glomerular staining was excluded from analysis.
**Diagnosis**

Patients with thin glomerular basement membrane disease (n=23) were compared with patients with chronic ischemic renal damage (n=22), IgA nephropathy (n=18), and focal segmental sclerosing glomerulonephritis (n=8). Other diagnoses could not be compared because of an insufficient number of patients. No significant differences in MR, sgk1, TGFβ-1, or IL-6 mRNA expression were found between these groups. Compared with patients with thin glomerular basement membrane disease, patients with focal segmental sclerosing glomerulonephritis had significantly increased MCP-1 mRNA expression (Δct, 9.57±4.09 versus 13.50±2.37; P<0.01) and urinary MCP-1 excretion (Δct, 661±427 versus 119±87 pg/mg creatinine; P<0.001).

**Proteinuria**

Patients with no albuminuria had the highest creatinine clearance (88.3±29.1 mL/min) compared with those with microalbuminuria (59.5±40.1 mL/min; P<0.005), moderate albuminuria (64.8±36.5 mL/min; P<0.005), and heavy albuminuria (50.0±31.7 mL/min; P<0.001). They also had the lowest blood pressure (systolic: 127.6±18.4 versus 135.3±15.2 mm Hg, P=NS; 143.1±14.1 mm Hg, P<0.005; and 144.0±17.1 mm Hg, P<0.05; diastolic: 71.0±10.4 versus 74.2±11.0 mm Hg, P=NS; 79.1±8.6 mm Hg, P<0.01; and 78.1±10.1 mm Hg, P=NS, respectively).

Patients with heavy albuminuria had the highest index of chronic damage (Figure 2A) and the least staining for CD34 (Figure 2B) compared with those with no albuminuria, microalbuminuria, and moderate albuminuria. Patients with heavy albuminuria had also the highest urinary MCP-1 protein concentrations (Figure 2C) and showed the most staining for macrophages (Figure 2D) compared with the other groups.

A 4.6-fold increase in MR mRNA expression was observed in patients with heavy albuminuria (Δct, 14.8±2.4) compared with no albuminuria (Δct, 17.0±3.3; P<0.05), microalbuminuria (Δct, 18.1±3.7; P<0.01), and moderate albuminuria (Δct, 17.2±2.0; P<0.01) (Figure 3A). A 2- and 3.4-fold increase in sgk1 mRNA expression was seen in those with moderate and heavy albuminuria (Δct, 11.7±1.2 and 11.0±2.9, respectively) compared with no albuminuria (Δct, 12.8±2.4; both P=NS) and microalbuminuria (Δct, 14.1±3.6; both P<0.05) (Figure 3A).

A 7.1-fold increase in MCP-1 mRNA expression was observed in patients with heavy albuminuria (Δct, 10.2±4.3) compared with no albuminuria (Δct, 13.7±3.2; P<0.05), microalbuminuria (Δct, 13.9±2.8; P<0.005), and moderate albuminuria (Δct, 14.0±2.5; P<0.05) (Figure 3B).

The expression of cytokines and profibrotic factors was increased in patients with heavy albuminuria. TGFβ-1 was increased 3.8-fold in heavy albuminuria (Δct, 13.9±2.9) compared with no albuminuria (Δct, 15.9±2.4; P<0.05), microalbuminuria (Δct, 17.6±5.1; P<0.05), and moderate albuminuria (Δct, 16.7±2.6; P<0.05). IL-6 was increased 2.7-fold (Δct, 21.7±2.5) compared with no albuminuria (Δct, 23.2±1.7; P<0.05), microalbuminuria (Δct, 23.9±2.6; P<0.05), and moderate albuminuria (Δct, 25.4±2.3; P<0.005) (Figure 3B).

MR mRNA expression showed a highly significant positive correlation with sgk1 mRNA expression (Figure 4). MCP-1, TGFβ-1, and IL-6 mRNA expression also positively correlated with MR mRNA expression (Figure 4) and sgk1 mRNA expression (Figure 5).

Serum aldosterone concentrations were not significantly different between the proteinuric groups: no albuminuria, 347±232 pmol/L; microalbuminuria, 455±256 pmol/L; moderate albuminuria, 474±349 pmol/L; and heavy albuminuria, 284±227 pmol/L.

**Localization of the MR**

In normal kidney, MR immunoreactivity was found predominantly in nuclei with some cytoplasmatic staining in cells of distal convoluted tubules and collecting tubules. No staining
was found in proximal tubules. Staining for MR was detected patchily in smooth muscle cells of arteries and veins and in the cells of the macula densa. Staining for MR in renal biopsies was detected in the same sites as in normal kidney. In general, the intensity of staining in distal tubules and collecting ducts was stronger in biopsies of patients with heavy proteinuria than in biopsies of patients with no proteinuria (Figure 6A and 6B). In addition, MR immunoreactivity was seen in some interstitial cells (Figure 6C).

**Discussion**

Patients with hyperaldosteronism have a higher incidence of left ventricular hypertrophy, albuminuria, and stroke than do patients with essential hypertension. Experimental animal models reveal the pathophysiological sequence after exposure to aldosterone and salt in the heart. Within weeks, there are increased production and activation of proinflammatory molecules, resulting in a histological picture of perivascular macrophage infiltration and inflammation. This is followed by increased interstitial collagen deposition, fibrosis, and ventricular hypertrophy. These events are prevented if an MR antagonist is used, with clinical trials (RALES and EPHEUS) confirming the beneficial effect of MR blockade in heart failure. Adverse structural remodeling of kidneys or heart does not appear when aldosterone treatment is given with a salt-depleted diet.

Activation of the MR by aldosterone also contributes to kidney damage in experimental models of hypertension. In hypertensive rats, aldosterone treatment induces severe vascular and glomerular sclerosis, fibrinoid necrosis, and thrombosis. In addition, it causes interstitial leukocyte infiltration and tubular damage and results in an increase in albuminuria and proinflammatory molecules. Administration of MR blockers or aldosterone ablation by adrenalectomy attenuates renal injury and reduces albuminuria and renal expression of proinflammatory molecules in rats independently of blood pressure reduction. These observations support the renoprotective effects of MR antagonism in nephropathy.

Local and systemically acting factors, notably MCP-1, TGFβ-1, and IL-6, have been associated with progressive renal injury. A prominent feature throughout the inflammatory process is the presence of leukocytes at sites of injury. MCP-1 is expressed by tubular epithelial and peritubular capillary endothelial cells, and levels of expression correlate with interstitial macrophage infiltration and fibrosis, suggesting that MCP-1 has a central role in directing macrophage recruitment to tissue sites.

In our study, serum aldosterone concentrations but not MR expression increased with loss of renal function compatible...
with an increased mineralocorticoid effect in patients with poor renal function. Other studies have described elevated serum aldosterone concentrations in patients with chronic renal disease. Patients with high aldosterone concentrations also showed a higher index of chronic damage than patients with low aldosterone concentrations, in keeping with rodent data. The renal expression of MCP-1 mRNA inversely correlated with renal function in agreement with urinary MCP-1 excretion in patients with chronic progressive renal disease. Additionally, MCP-1 expression correlated with expression of the pan-macrophage antigen CD68, supporting the hypothesis that MCP-1 plays a role in directing macrophages to tissue sites.

In human and rodents, proteinuria seems to be a major risk factor and pathological stimulus of renal inflammation. In experimental animal models, the deleterious effects of aldosterone seemed to be related to proteinuria, with MR blockade causing a decrease in proteinuria. Preliminary data suggest that spironolactone decreases proteinuria in patients with chronic renal disease and those with type 2 diabetes mellitus and early nephropathy, but the mechanisms are unclear. In our study, patients with heavy albuminuria presented with the highest index of chronic damage, the lowest staining for CD34, and the worst renal survival (end points were doubling of serum creatinine concentrations or dialysis). This group of patients displayed the highest renal MCP-1 mRNA expression and urinary MCP-1 protein excretion and consequently high macrophage invasion as shown by staining for CD68. Bearing in mind first that aldosterone infusion in rats led to increased renal proinflammatory gene expression such as MCP-1 and second that this inflammation was attenuated by eplerenone, we analyzed mRNA and protein expression of MR and mRNA expression of the MR target gene sgk1 in the renal biopsies. Serum aldosterone concentrations did not differ significantly between the groups with different levels of albuminuria, but patients with heavy albuminuria showed a marked increase (5-fold) in renal MR mRNA expression compared with patients with no or moderate albuminuria. The correlation with and concomitant increase (2.5-fold) in sgk1 mRNA expression in these patients suggest that the increase in MR effector systems is both real and functional. Immunohistochemistry data confirmed that the increase in MR expression is within the distal nephron, although some interstitial cells stained for MR. In keeping with the association found by Blasi et al that aldosterone regulates MCP-1 expression, probably via the MR, we found a significant correlation between MR and MCP-1 mRNA expression. This finding underpins the possible direct interaction between those 2 systems. We cannot conclude whether the induction of MCP-1 is mediated via sgk1 or possibly via other unknown MR target genes; this
subject needs further clarification. There is also the possibility of an indirect effect of aldosterone leading to a proinflammatory vascular phenotype of the kidneys and heart. For example, increased aldosterone is associated with activation of circulating immune cells that are involved in a proinflammatory/fibrogenic vascular phenotype of the heart and systemic organs. Increased aldosterone is associated with activation of circulating immune cells that are involved in a proinflammatory/fibrogenic vascular phenotype of the heart and systemic organs.

In addition to systemic aldosterone levels, local tissue-specific aldosterone production might play a role at least in cardiac fibrosis, although aldosterone synthase-transgenic mice showed no structural or myocardial alterations. This highlights that in vitro studies with aldosterone often have not been consistent and reproducible.

Inflammation is an important contributor to renal damage. We investigated the expression of further proinflammatory cytokines in renal biopsies. The cytokine IL-6 is associated with subsequent cardiovascular mortality and progression of vascular disease and correlates with the degree of mesangial hyperproliferation, tubular atrophy, and the intensity of interstitial infiltration. Furthermore, it is known to directly induce MCP-1 expression. Our patients with heavy albuminuria showed a significant increase in IL-6 mRNA expression that may explain, in part, the increased MCP-1 expression. Blasi et al showed that renal IL-6 expression is increased in rats treated with aldosterone. We did find a correlation between MR and sgk1 expression and IL-6 expression, but a causative relationship remains to be established.

TGF-1 is an important cytokine that promotes fibroblast differentiation and proliferation, upregulates collagen synthesis and deposition, and inhibits matrix metalloproteinase collagenases. In proteinuric rats, TGF-1 production from interstitial macrophages correlated with inflammatory infiltration. TGF-1 is increased by treatment with aldosterone, and in rats with renal fibrosis, expression could be
blocked by treatment with spironolactone. Human studies on TGF-β-1 are scarce. Increased TGFβ-1 expression has been reported in human diabetic patients and in patients with nephrotic syndrome. Here, we found a significant correlation between renal MR and renal TGF-β-1 mRNA expression, supporting this link, and observed a 3-fold increase in renal TGFβ-1 mRNA expression in patients with heavy albuminuria. Therefore, we believe MR via sgk1 or as-yet-unknown MR target genes regulate the expression of several inflammatory cytokines. Preliminary but small clinical trials have analyzed the effect of MR antagonists on chronic renal injury, but there is now an obvious need for larger trials with additional end points besides proteinuria. Hyperkalemia has been raised as a potential deleterious side effect, particularly in patients with reduced renal function, heart failure, or diabetes, but careful titration and the use of lower doses of MR antagonists minimize this risk.

In conclusion, in patients with heavy albuminuria, the expression of MR and sgk1 mRNA is significantly increased, suggesting a direct receptor-mediated activation of mineralocorticoid action. This group of patients is at the highest risk for progression of renal damage and failure as indicated by high renal MCP-1 mRNA and urinary levels, high macrophage invasion, and a high index of chronic damage. Because of an increase in MR effector mechanisms, expression of inflammatory mediators such as IL-6 and TGFβ-1 are enhanced and further promote renal inflammation. These observations support the further evaluation of MR antagonists in patients with renal disease, particularly those with severe proteinuria.

Acknowledgments
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References


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