Monocyte Infiltration and Adhesion Molecules in a Rat Model of High Human Renin Hypertension

Eero M.A. Mervaala, Dominik N. Müller, Joon-Keun Park, Folke Schmidt, Matthias Löhn, Volker Breu, Duska Dragun, Detlev Ganten, Herman Haller, Friedrich C. Luft

Abstract—Hypertension and kidney damage in the double transgenic rat (dTGR) harboring both human renin and human angiotensinogen genes are dependent on the human components of the renin angiotensin system. We tested the hypothesis that monocyte infiltration and increased adhesion molecule expression are involved in the pathogenesis of kidney damage in dTGR. We also evaluated the effects of long-term angiotensin-converting enzyme (ACE) inhibition, AT1 blockade, and human renin inhibition on monocyte recruitment and inflammatory response in dTGR. Systolic blood pressure and 24-hour albuminuria were markedly increased in 7-week-old dTGR as compared with age-matched normotensive Sprague Dawley rats. We found a significant monocyte/macrophage infiltration in the renal perivascular space and increased expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in the interstitium, intima, and adventitia of the small renal vessels. The expression of plasminogen activator inhibitor-1 and fibronectin in the kidneys of dTGR were increased and distributed similarly to ICAM-1. In 4-week-old dTGR, long-term treatment with ACE inhibition (cilazapril), AT1 receptor blockade (valsartan), and human renin inhibition (RO 65-7219) each drug 10 mg/kg by gavage once a day for 3 weeks) completely prevented the development of albuminuria. However, only cilazapril and valsartan were able to decrease blood pressure to normotensive levels. Interestingly, the drugs were all equally effective in preventing monocyte/macrophage infiltration and the overexpression of adhesion molecules, plasminogen activator inhibitor-1, and fibronectin in the kidney. Our findings indicate that angiotensin II causes monocyte recruitment and vascular inflammatory response in the kidney by blood pressure–dependent and blood pressure–independent mechanisms. ACE inhibition, AT1 receptor blockade, and human renin inhibition all prevent monocyte/macrophage infiltration and increased adhesion molecule expression in the kidneys of dTGR. (Hypertension. 1999;33[part II]:389-395.)

Key Words: angiotensin II • intercellular adhesion molecule-1 • vascular cell adhesion molecule-1 • plasminogen activator inhibitor-1 • fibronectin • renin

Hypertension is a major risk factor for renal injury. However, the mechanisms underlying the development and progression of hypertension-induced kidney damage are incompletely understood. There is growing evidence that vascular inflammatory responses and interstitial accumulation of extracellular matrix proteins are involved in the pathogenesis.1,2 Moreover, both experimental and clinical studies revealed that angiotensin II (Ang II), the key effector of the local and circulating renin-angiotensin system (RAS), plays a central role in the pathogenesis of hypertension-induced end-organ damage (for reviews see References 3 and 4). The mechanisms of Ang II–induced hypertension and renal damage are generally attributed to direct vasoconstriction, enhancement of sympathetic adrenergic transmission, and increased secretion of aldosterone and endothelins.3,4 However, recent studies have suggested that Ang II could also induce end-organ damage and endothelial dysfunction by production of oxidative stress5 and by enhancing leukocyte adhesion on endothelial cells.6 In rats, physiological and pharmacological experiments aimed at exploring the role of the RAS in hypertension is hampered by the species specificity of the renin-angiotensin interaction. The hypertension of double transgenic rat (dTGR) harboring the human renin and human angiotensinogen genes is dependent on the human RAS components.7 These rats offer a unique opportunity to study the human RAS and pharmacologically induced changes of the human RAS in an experimental animal model. We tested the hypothesis that monocyte infiltration and increased adhesion molecule expression are involved in the pathogenesis of kidney damage in dTGR. We
also evaluated the effects of long-term angiotensin-converting enzyme (ACE) inhibition, AT1 blockade, and human renin inhibition on monocyte recruitment and inflammatory response in dTGR. We showed that Ang II caused moderate hypertension and a progressive increase in albuminuria, induced monocyte/macrophage infiltration, and led to extensive adhesion molecule expression in the kidney that was followed by an increased expression of plasminogen activator inhibitor-1 (PAI-1) and fibronectin. Cilazapril, valsartan, and the human renin inhibitor were equally effective in preventing vascular inflammatory response, although only cilazapril and valsartan were able to decrease blood pressure to normotensive levels. Our findings suggest that Ang II causes monocyte/macrophage infiltration and vascular inflammatory responses in the kidneys by blood pressure–dependent and blood pressure–independent mechanisms.

### Methods

Experiments were conducted in 73 4-week-old male dTGR (body weight 58±1 g) and in 15 normotensive Sprague-Dawley rats (SD) (53±2 g). The dTGR line and characteristics are described in detail elsewhere.

Briefly, the human renin construct used to generate transgenic animals made up the entire genomic human renin gene (10 exons and 9 introns), with 3.0 kB of the 5' promoter region and 1.2 kB of 3' additional sequences. The human angiotensinogen construct made up the entire human angiotensinogen gene (5 exons and 4 introns), with 1.3 kB of 5' flanking and 2.4 kB of 3' flanking sequences. The rats were purchased from Biological Research Laboratories Ltd (Füllinsdorf, Switzerland) and were allowed free access to standard 0.3% sodium rat chow (SSNFF Spezialitäten GmbH) and drinking water. The procedures and experimental protocols were approved by the local Council on Animal Care, whose standards correspond to those of the American Physiological Society. Four-week-old dTGR were divided into 4 groups: (1) control group (n=26), (2) human renin inhibitor group (RO 65-7219) (n=15), (3) cilazapril group (n=16), and (4) losartan group (n=16).

The drugs were given for 3 weeks by gavage once per day (10 mg/kg). Control dTGR and SD rats received vehicle (1% sodium carboxymethylcellulose). Systolic blood pressure was measured weekly by tail-cuff method under light ether anesthesia 20 hours after the last drug dose, starting at the age of 5 weeks. Urine samples were collected over a 24-hour period by metabolic cages at 5, 6, and 7 weeks. Ten dTGR and 5 SD rats were killed under pentobarbital anesthesia (65 mg/kg IP) at the age of 5 weeks, whereas all other rats were killed at age 7 weeks. Whole blood samples for flow cytometry were taken into EDTA (4.5 mmol/L) tubes. The kidneys were washed with ice-cold saline and weighed. For immunohistochemistry, the kidney was cut sagittally, snap-frozen in isopentane (−35°C), and stored at −80°C. Frozen kidneys were processed and semiquantitative scoring performed as described in detail previously.

Monoclonal antibodies against rat monocytes/macrophages (ED1) (Serotec, Oxford, England), intercellular adhesion molecule-1 (ICAM-1) (1A29) (R&D Systems), αβ4 integrin (WT01), vascular cell adhesion molecule-1 (VCAM-1) (51-10C9) (Pharmingen, San Diego, Calif), αβ2 integrin (TA-4) (Pharmingen), and polyclonal antibodies against PAI-1 (American Diagnostica, Greenwich, Conn) and fibronectin (Paez+Lorei, Frankfurt, Germany) were used. Light microscopic techniques that we used are described in detail elsewhere.

The kidney samples were examined without knowledge of the rat’s identity group. Fluorescence sorting analysis from circulating blood cells was carried out according to the instructions of the manufacturer (Becton Dickinson) with fluorochrome-conjugated monoclonal antibodies against rat αβ1 integrin (CD11a) (Serotec, Oxford, England). Quantitative determination of the monocyte chemotactic protein-1 (MCP-1) concentration in the urine and plasma was performed with a commercially available rat MCP-1 ELISA kit (Pharmingen). Urinary MCP-1 concentrations were normalized to urine creatinine.

Data are presented as mean±SEM. Statistically significant differences in mean values were tested by 2-way ANOVA for repeated measures and the Tukey’s multiple range test. A value of P<0.05 was considered statistically significant. The data were analyzed with SYSTAT statistical software.

### Results

Cilazapril and valsartan treatment but not treatment with human renin inhibitor decreased blood pressure to levels observed in normotensive SD rats (Figure 1A). Systolic blood pressure of the human renin inhibitor group was significantly decreased only when measured 4 hours after drug administration (151±8 vs 178±4 mm Hg, P<0.05). There was a progressive increase in blood pressure and albuminuria in untreated dTGR. All drug treatments completely prevented development of albuminuria, whereas only valsartan and cilazapril normalized blood pressure. *P<0.05 dTGR control group vs cilazapril, valsartan, and SD groups; 0.05 renin inhibitor group vs cilazapril, valsartan, and SD groups; dTGR control group vs renin inhibitor group.

Figure 1. Systolic blood pressure (A) and 24-hour urinary albumin excretion in dTGR and SD rats. dTGR indicates controls without treatment; RI, human renin inhibitor group; VAL, valsartan group; CILA, cilazapril group, SD, normotensive Sprague-Dawley rats. There was a progressive increase in blood pressure and albuminuria in untreated dTGR. All drug treatments completely prevented development of albuminuria, whereas only valsartan and cilazapril normalized blood pressure. *P<0.05 dTGR control group vs cilazapril, valsartan, and SD groups; 0.05 renin inhibitor group vs cilazapril, valsartan, and SD groups; dTGR control group vs renin inhibitor group.
intimal and medial thickening and hyaline deposits. The tubules were frequently swollen and filled with proteinaceous material, whereas the glomeruli were usually not affected (data not shown). Long-term treatment with human renin inhibitor (Figures 2 through 5), cilazapril, and valsartan all prevented monocyte/macrophage infiltration and adhesion molecule expression in the kidneys (Table). The drug treatments also blocked the morphological changes observed in untreated dTGR (data not shown).

MCP-1 concentration in urine was increased ≈130% in dTGR compared with normotensive SD rats (Figure 6A).

Human renin inhibitor, cilazapril, and valsartan decreased urinary MCP-1 concentration to levels found in SD rats. There was no difference between the treatment groups in plasma MCP-1 concentrations (Figure 6B).

**Discussion**

The dTGR harboring both human renin and human angiotensinogen genes feature relatively moderate hypertension with severe vascular lesions in the kidneys and the heart.7 We showed recently that pressure-natriuresis-diuresis curves in

<table>
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<th>dTGR Controls, 5 Weeks</th>
<th>DTGR Controls, 7 Weeks</th>
<th>dTGR + RI, 7 Weeks</th>
<th>DTGR + CILA, 7 Weeks</th>
<th>dTGR + VAL, 7 Weeks</th>
<th>SD Controls, 5 Weeks</th>
<th>SD Controls, 5 Weeks</th>
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<td>−/(●)</td>
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<td>ICAM-1</td>
<td>●●●●(●)</td>
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<td>α₁β₂ Integrin</td>
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<td>VCAM-1</td>
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- indicates no staining; ●, very weak staining; ●●, weak staining; ●●●, moderate staining; ●●●●, strong staining; and ●●●●●, very strong staining.
dTGR are shifted toward higher renal perfusion pressure ranges by Ang II–dependent mechanisms inherent to the kidneys themselves. The main findings here were that dTGR developed severe albuminuria that was associated with pronounced monocyte/macrophage infiltration and renal expression of surface adhesion molecules ICAM-1 and VCAM-1. We also demonstrated increased expression of $\alpha_2\beta_1$ integrin and $\alpha_4\beta_1$ integrin, the counterreceptors for ICAM-1 and VCAM-1, on penetrating monocytes/macrophages as well as circulating inflammatory cells. Whereas ICAM-1 expression was constantly increased over time, the increased expression of VCAM-1 was most prominent in the early phase of renal injury. The monocyte recruitment in the vascular wall was accompanied by increased PAI-1 expression in the same areas and accumulation of extracellular matrix protein. Human renin inhibitor, ACE inhibitor, and AT1 receptor antagonist treatment prevented the development of albuminuria, monocyte/macrophage infiltration, and vascular inflammatory response. Our findings indicate that the development of kidney injury in dTGR is dependent on Ang II and is associated with pronounced vascular inflammatory response and overexpression of adhesion molecules ICAM-1 and VCAM-1.

We sought to elucidate mechanisms involved in the pathogenesis of renal injury in dTGR. Long-term Ang II infusion in normotensive rats results in moderate hypertension and marked vascular, glomerular, and tubulointerstitial injury and interstitial fibrosis. In dTGR, arterial hypertension induced by the human components of the RAS causes mechanical stress to endothelial and smooth muscle cells that may in part account for the development of renal vascular damage. Even though hemodynamic forces alone are capable of inducing vascular remodeling and subsequent end-organ damage, blood pressure–independent mechanisms mediated by high Ang II levels are also likely to be involved. Our finding that human renin inhibitor completely prevented the development of albuminuria as well as monocyte/macrophage infiltration and overexpression of adhesion molecules in the kidneys with only a partial decrease in blood pressure supports this notion. We would like to underline the fact that our monoclonal antibody used for the detection of infiltrating cells detects mainly rat monocytes and macrophages. However, it is likely that other inflammatory cells, such as neutrophils and myofibroblasts, are also involved in the inflammatory response in dTGR. Furthermore, even though we were able to demon-

![Figure 3. Representative immunohistochemical photomicrographs of $\alpha_2\beta_1$ integrin (WT0.1) in kidney (top) and fluorescence sorting analysis of $\alpha_4\beta_1$ integrin (LFA-1) (CD11a) from circulating blood cells (bottom) of SD rat, dTGR, and human renin inhibitor–treated dTGR. FSC-H indicates forward scattering-high.](image-url)
strate a close association between monocyte recruitment, overexpression of adhesion molecules, and the development of albuminuria, the present study does not give direct evidence that inflammatory cell infiltrate mediates renal injury in dTGR.

The underlying mechanisms of the Ang II–dependent vascular inflammatory response and expression of adhesion molecules are only poorly understood. McCarron et al.11,12 have shown previously that cytokine or endotoxin-stimulated ICAM-1 expression and monocyte adhesion is more intense on endothelial cells derived from SHR compared with cells from WKY rats, indicating that hypertension may enhance responsiveness of endothelium to factors that promote monocyte adhesion. However, inconsistent with this report, Komatsu et al.13 demonstrated recently that constitutive ICAM-1 expression in the microvasculature does not differ between WKY and SHR in vivo. Ang II induces leukocyte adhesion on human endothelial cells at least in vitro and modulates the expression of E-selectin, an adhesion molecule that has been implicated in initiating cellular contact between leukocytes and endothelium and that plays a central role in the leukocyte “rolling” phenomenon under blood-flow conditions.6 The present study describes that Ang II induces surface molecules ICAM-1 and VCAM-1 in the vascular endothelium as well as their counterreceptors \( \alpha_\beta \) integrin and \( \alpha_4 \beta_1 \) integrin in the circulating and infiltrating monocytes. Consistent with our findings, Mai et al.2 have demonstrated previously that ICAM-1 expression increases progressively in kidneys exposed to high blood pressure in the high renin phase of 2-kidney 1-clip Goldblatt model. Here we also showed that vascular inflammatory response as well as the overexpression of the adhesion molecules in the kidneys can be prevented effectively by drugs interfering the RAS.

We observed copious PAI-1 expression in the kidneys of dTGR. PAI-1 is a major physiological inhibitor of the plasminogen activator/plasmin system, a key regulator of fibrinolysis and extracellular matrix turnover.14 Activation of the RAS can disturb the balance of the fibrinolytic system by stimulating excess production of PAI-1 and thereby increasing the risk of thrombotic events. We have not studied this pathway in greater detail; however, untreated dTGR given high salt (unpublished observations) show accentuated renal damage with microthrombi and fibrin deposition. These same mechanisms are probably active on a smaller scale here. RAS blockade ameliorates these mechanisms.

Our double transgenic rat model of high human renin hypertension allowed us to examine species-specific human renin inhibitor in rats. At the dose tested, the human renin inhibitor RO 65-7219 decreased blood pressure significantly less than cilazapril and valsartan. In fact, the modest blood pressure lowering effect by RO 65-7219 was observed only after 4 hours. Previous pharmacokinetic studies have shown

Figure 4. Representative immunohistochemical photomicrographs of VCAM-1 (51-10C9) (top) and its counterreceptor \( \alpha_\beta \) integrin (TA-4) (bottom) in kidneys of SD rat, dTGR, and human renin inhibitor–treated dTGR.
that human renin inhibitors have very high hepatic clearance and low oral efficacy. An unfavorable pharmacokinetic profile is likely to explain the lack of any significant antihypertensive effect by RO 65-7219 in dTGR. However, the uptake of human renin inhibitors by the kidney may act as reservoir for the drug, resulting in the prolonged duration of pharmacological activity locally. We conclude that moderate hypertension and severe albuminuria is highly dependent on Ang II in the dTGR model. Ang II is able to induce pronounced monocyte recruitment and vascular inflammatory responses in the kidney by blood pressure–dependent and blood pressure–independent mechanisms. ACE inhibition, AT₁ receptor blockade, and human renin inhibition prevent monocyte/macrophage infiltration and increased adhesion molecule expression in dTGR.

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