Involvement of connective tissue growth factor in human and experimental hypertensive nephrosclerosis

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Abstract

Background/Aims: Connective tissue growth factor (CTGF; CCN2) has been implicated as a marker and mediator of fibrosis in human and experimental renal disease. Methods: We performed a comparative analysis of CTGF expression in hypertensive patients with and without nephrosclerosis, and in uninephrectomized and sham-operated spontaneously hypertensive rats (UNX-SHR and 2K-SHR). Results: Urinary and plasma CTGF were elevated in patients with hypertensive nephrosclerosis, and increased renal CTGF expression was mainly localized in podocytes. Accordingly, elevation of urinary, plasma, and tissue CTGF in UNX-SHR coincided and correlated with proteinuria, glomerulosclerosis, and tubulointerstitial fibrosis. Thirty-two weeks after uninephrectomy, mean glomerular CTGF mRNA expression was increased 1.3-fold over baseline, mainly due to 1.7-fold higher expression in glomeruli undergoing sclerosis. In parallel, tubulointerstitial CTGF and α-smooth muscle actin were upregulated in UNX-SHR. CTGF was increased in the media of arcuate and interlobar arteries, while arterioles remained negative. Conclusions: Glomerulosclerosis, tubulointerstitial fibrosis, and arterial media hypertrophy lesions of hypertensive nephrosclerosis are all characterized by increased CTGF tissue expression, which is associated with a concomitant increase in CTGF in blood and urine. These findings identify CTGF as a promising biomarker for progression of hypertensive nephrosclerosis, and as a likely key factor in the pathogenesis of this disease.
Introduction

Hypertensive nephrosclerosis is a major cause of renal morbidity and mortality, which underscores the importance of clarifying the mechanisms involved in hypertensive renal injury [1]. As in other forms of renal injury, development of irreversible and progressive renal scarring in hypertension is probably driven by a number of proinflammatory and fibrogenic cytokines, including transforming growth factor (TGF)-β [2,3]. Effects of TGF-β on renal cells appear to be mediated at least in part by connective tissue growth factor (CTGF; CCN2) [4–9].

Previously, we have reported that CTGF expression is markedly increased in human glomerular and tubulointerstitial lesions associated with cellular proliferation, but not in non-inflammatory lesions with only proteinuria. Furthermore, expression of CTGF mRNA was strongly correlated with the extent of tubulointerstitial fibrosis, across different renal diseases [10]. Recently, CTGF was reported to be increased in the aorta and the kidney of spontaneously hypertensive rats (SHR) [11,12]. However, renal damage in SHR was limited and no glomerulosclerosis was observed in these latter studies. It is known that uninephrectomy (UNX) in SHR (UNX-SHR) results in accelerated severe systemic hypertension, and progressive glomerulosclerosis and tubulointerstitial fibrosis similar to the late stage of human hypertensive nephrosclerosis. This makes UNX-SHR an appropriate model for long-standing hypertension [13,14].

To investigate the possible involvement of CTGF in hypertensive nephrosclerosis, we analyzed CTGF tissue, plasma, and urine levels in hypertensive human patients with and without renal dysfunction, and in SHR and UNX-SHR.

Materials and Methods

Patient Profile and Collection of Urine

We measured urinary CTGF in 40 patients with nephrosclerosis (n = 21) or hypertension stage 1 (n = 19), and in 28 healthy controls. All had provided their informed consent, and the study was performed according to the principles of the Declaration of Helsinki and approved by an ethical committee. Patient profiles are given in Table 1. Patients with stage 1 hypertension were previously diagnosed with systolic blood pressure between 140 and 159 mm Hg. Table 1 shows blood pressure values at the time when plasma and urine were collected for measurement of CTGF. Diagnosis of hypertensive nephrosclerosis was based on clinical features, i.e. the presence of long-standing hypertension with renal insufficiency in the absence of pyelonephritis, primary glomerulonephritis, and metabolic diseases. Because all patients with hypertensive nephrosclerosis showed characteristic clinical features, no confirmation by renal biopsy was indicated in our study. For urinalysis, early morning voided urine samples were used. For determination of urinary CTGF, 9.5 ml of fresh urine was mixed with 0.5 ml of 0.5 mol/l EDTA and centrifuged at 2,000 r.p.m. for 10 min and stored at −70°C
ELISA for Plasma and Urinary CTGF

CTGF levels in plasma and urine of patients and rodents were determined by sandwich ELISA (FibroGen, San Francisco, Calif., USA) [16–18]. Levels of plasma CTGF are expressed as picomoles per liter. Urinary CTGF excretion is expressed as picomoles per milligram creatinine. The detection limit of these assays is 4 pmol/l.

Animals and Experimental Protocol

SHR (Harlan-Olac, UK) were housed in standard cages in a room with constant temperature, on a 12-hour light-dark cycle. Rats were fed a standard pellet laboratory chow and had free access to water. The experiments were performed with the approval of the Experimental Animal Ethics Committee of the University of Utrecht. We designated 5 groups as summarized in Table 2. Rats in group I (2K0W) were killed at 8 weeks. Rats in group III (1K17W) and group V (1K32W) underwent right nephrectomy by sterile surgery at 8 weeks under fentanyl/diazepam anesthesia and were killed after 17 and 32 weeks, respectively. Rats in group II (2K17W) and group IV (2K32W) were sham-operated (exposure of right kidney) and killed after 17 and 32 weeks, respectively.

Renal Function and Plasma Lipids

Urine was collected before surgery to determine urinary protein excretion. Creatinine levels in plasma and urine were determined colorimetrically. Total plasma cholesterol and triglycerides were determined enzymatically. Systolic blood pressure was measured before sacrifice using the tail cuff method.

Renal Morphology, Immunohistochemistry, and in situ Hybridization

Animals were sacrificed under pentobarbital anesthesia. Subsequently, the left kidney was decapsulated, removed, weighed and processed for morphology, immunohistochemistry (IHC), in situ hybridization (ISH), and RT-PCR. Monoclonal antibody 1A4 (Dako, Denmark) was used for visualization of α-smooth muscle actin (α-SMA) expression in paraffin sections. Anti-TGF-β1, 2, and 3 polyclonal antibody (R&D Systems, UK) was applied on frozen sections. For CTGF IHC on human and mice renal tissue, paraffin sections were pretreated with protease XXIV (Sigma), and stained with monoclonal anti-CTGF antibody (FibroGen) [18, 19]. A digoxigenin-labeled CTGF probe was used for ISH, as described in detail previously [10, 20].
Quantification of IHC, ISH and Sclerotic Lesions

Fractions of the glomerular surface areas with positive staining for CTGF mRNA (cross-sections of 20 different glomeruli/rat), and α-SMA protein (20 different tubulointerstitial areas of 1 mm²) were measured using NIH Image software [20]. The presence of segmental or global sclerotic glomeruli was assessed on PAS-stained sections (average of 181 ± 13 glomeruli/rat). CTGF mRNA expression in the arteries was scored as: 0 = no staining; 1 = weak staining; 2 = segmental or diffuse weak staining, and 3 = diffuse staining. For arterioles, interlobular arteries, and interlobar and arcuate arteries, the average CTGF expression score was determined in more than 10 cross-sections/kidney.

Statistical Analysis

Values are expressed as mean ± SD. Comparison among groups of patients or among groups of animals was performed by one-way analysis of variance (ANOVA) followed by Scheffé’s F test or Dunnett’s method. Forward stepwise regression analysis was used to compare urinary CTGF level with proteinuria and plasma CTGF level with proteinuria or pathological parameters. Correlation coefficients were calculated by Pearson analysis. All analyses were performed using SPSS software (Chicago, Ill., USA).

Table 2. Characteristics of the SHR model

<table>
<thead>
<tr>
<th></th>
<th>I (2K0W)</th>
<th>II (2K17W)</th>
<th>III (1K17W)</th>
<th>IV (2K32W)</th>
<th>V (1K32W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of study, weeks</td>
<td>0</td>
<td>17</td>
<td>17</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Strain</td>
<td>SHR</td>
<td>SHR</td>
<td>SHR</td>
<td>SHR</td>
<td>SHR</td>
</tr>
<tr>
<td>2K/UNX</td>
<td>2K</td>
<td>2K</td>
<td>UNX</td>
<td>2K</td>
<td>UNX</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>4</td>
<td>8</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Proteinuria, mg/day</td>
<td>19 ± 8</td>
<td>38 ± 8</td>
<td>48 ± 16 a</td>
<td>34 ± 6</td>
<td>141 ± 77 b</td>
</tr>
<tr>
<td>Urine volume, ml/day</td>
<td>9.4 ± 2.6</td>
<td>12.0 ± 4.1</td>
<td>21.4 ± 14.1 a</td>
<td>14.5 ± 7.5</td>
<td>16.6 ± 5.2</td>
</tr>
<tr>
<td>Plasma creatinine, μmol/l</td>
<td>52 ± 4</td>
<td>46 ± 2</td>
<td>56 ± 9</td>
<td>47 ± 10</td>
<td>54 ± 5</td>
</tr>
<tr>
<td>Creatinine clearance, ml/min</td>
<td>2.2 ± 0.33</td>
<td>2.3 ± 0.29</td>
<td>2.0 ± 0.34 a</td>
<td>2.3 ± 0.41</td>
<td>2.0 ± 0.31</td>
</tr>
<tr>
<td>Systolic pressure, mm Hg</td>
<td>170 ± 0.15</td>
<td>248 ± 3 c</td>
<td>252 ± 3 c</td>
<td>250 ± 4 c</td>
<td>251 ± 5 c</td>
</tr>
<tr>
<td>Plasma cholesterol, mmol/l</td>
<td>1.60 ± 0.15</td>
<td>1.13 ± 0.25</td>
<td>1.35 ± 0.17</td>
<td>1.39 ± 0.13</td>
<td>1.93 ± 0.47 d</td>
</tr>
<tr>
<td>Plasma triglyceride, mmol/l</td>
<td>0.50 ± 0.12</td>
<td>0.65 ± 0.13</td>
<td>0.95 ± 0.21 e</td>
<td>0.53 ± 0.20</td>
<td>1.11 ± 0.36 c</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>191 ± 11</td>
<td>318 ± 43</td>
<td>427 ± 16</td>
<td>397 ± 24</td>
<td>445 ± 20 e</td>
</tr>
<tr>
<td>Left kidney weight, g</td>
<td>0.69 ± 0.05</td>
<td>1.32 ± 0.15</td>
<td>2.14 ± 0.10 b</td>
<td>1.43 ± 0.13</td>
<td>2.45 ± 0.37 e</td>
</tr>
<tr>
<td>Left kidney weight, g / kg body weight</td>
<td>3.59 ± 0.20</td>
<td>4.63 ± 0.17</td>
<td>5.01 ± 0.17 b</td>
<td>3.60 ± 0.32</td>
<td>5.50 ± 0.83</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. SHR = Spontaneously hypertensive rat; UNX = uninephrectomized; 2K = sham-operated. *p < 0.05 versus II; p < 0.001 versus IV; p < 0.0001 versus I; p < 0.05 versus IV; p < 0.01 versus II; p < 0.005 versus IV; #p < 0.01 versus IV; $p < 0.0001 versus II; p < 0.0001 versus IV.

Results

CTGF in Plasma, Urine, and Kidney of Human Hypertensive Patients

Patients with hypertensive nephrosclerosis had only slight proteinuria in spite of moderate renal dysfunction (table 1). Patients with hypertensive nephrosclerosis had elevated plasma CTGF levels (543.2 ± 335.8 pmol/l) as compared to control subjects and hypertensive patients with normal renal function (182.0 ± 123.1 and 285.5 ± 260.2 pmol/l, respectively; fig. 1A). Patients with hypertensive nephrosclerosis had elevated urine CTGF levels (1,113.1 ± 1,620.4 pmol/mg Cr) as compared to control and
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Fig. 1. Urine and plasma CTGF levels in human nephrosclerosis. CTGF levels were determined by sandwich ELISA. A Patients with hypertensive nephrosclerosis have elevated plasma CTGF levels as compared to control subjects and hypertensive patients with normal renal function. B Patients with hypertensive nephrosclerosis have elevated urine CTGF levels as compared to control and hypertensive patients. * p < 0.05 versus control and hypertension groups; # p < 0.005 versus hypertension group; ## p < 0.001 versus control group.

Fig. 2. Tissue CTGF expression in human nephrosclerosis. CTGF protein expression was assessed by immunohistochemistry on sections counterstained with PAS. A, B Weak CTGF staining of some podocytes in a renal biopsy from a non-hypertensive subject. C, D CTGF is strongly expressed in glomerular podocytes in a representative renal biopsy of a patient with hypertensive nephrosclerosis. Bar sizes: 100 μm (A, C), 20 μm (B, D).
hypertensive patients (73.0 ± 37.3 and 91.8 ± 47.8 pmol/mg Cr, respectively; fig. 1B). Figure 2 shows strong CTGF protein staining in glomerular podocytes of a patient with hypertensive nephrosclerosis.

**UNX-SHR Model**

Basic characteristics of SHR and UNX-SHR are summarized in table 2. Systolic blood pressure was increased with age and not affected by UNX. After 17 weeks, urinary protein excretion in group III (1K17W) was significantly higher than in group II (2K17W), and continued to increase 32 weeks after UNX (group V, 1K32W). Plasma creatinine levels and creatinine clearance were similar in all groups.

**Correlation of Urinary and Plasma CTGF with Functional Parameters**

Urinary CTGF correlated significantly with proteinuria in groups IV (2K32W) and V (1K32W; \( R^2 = 0.47, p < 0.01 \); fig. 3A). Plasma CTGF levels in groups III (1K17W) and V (1K32W) were significantly increased as compared to controls (309.5 ± 120.1 in group III and 308.1 ± 92.1 in group V vs. 152.5 ± 15.4 in group I, 200.7 ± 150.1 in group II and 146.9 ± 42.3 pmol/l in group IV, \( p < 0.05 \); fig. 3B). Also plasma CTGF correlated with proteinuria (\( R^2 = 0.33, p < 0.001 \); fig. 3C). In addition, plasma CTGF correlated with urinary CTGF excretion (\( R^2 = 0.46, p < 0.01 \)). No correlation was observed between urinary CTGF excretion and creatinine clearance (\( R^2 = 0.042, p = 0.48 \)).
Fig. 4. CTGF mRNA in glomerular lesions of control SHR and 32 weeks after UNX-SHR. CTGF mRNA was assessed by in situ hybridization. **A, B** In control kidney (group I, 2K0W), CTGF mRNA is expressed mainly by glomerular podocytes. In the tubulointerstitial area, CTGF is not detected. **C, D** CTGF mRNA is overexpressed in the glomeruli and fibrotic tubulointerstitial area of group V (1K32W). **E, F** In consecutive sections, Azan stain shows association of fibrosis with increased CTGF mRNA expression in glomerular podocytes and parietal epithelium, and in interstitial fibroblasts. Long arrow = Segmental adhesion and scar; short arrows = interstitial fibroblasts; open arrowheads = parietal epithelium; black arrowheads = visceral epithelium (podocytes). **G** Morphometric analysis of ISH for CTGF mRNA expression, immunohistochemistry (IHC) for glomerular α-SMA expression and percentage of glomeruli with FSGS lesions shows that mean glomerular CTGF expression was increased 1.3-fold to group I. Both CTGF and α-SMA are increased specifically in glomeruli with FSGS lesions (V-Scl.) as compared to normal appearing glomeruli of the same kidneys (V-non-Scl.). # p < 0.05; ## p < 0.01, group V versus group I; * p < 0.01, group V-Scl. versus group V-non-Scl.
Fig. 5. CTGF protein in control SHR and SHR 32 weeks after UNX. CTGF protein was assessed by immunohistochemistry. A, B In control SHR kidney (group I; 2K0W), CTGF protein is detected only in glomerular podocytes and weakly in medial smooth muscle cells of large arteries, but not in normal tubulointerstitial area. C, D CTGF protein expression is increased in the fibrotic tubulointerstitial area. Strong CTGF expression is detected in interstitial fibrotic lesions around atrophic tubules (short arrows), and also in some flattened tubular epithelial cells. However, the mononuclear infiltrating cells appear to be negative for CTGF. E, F In group V (1K32W), glomerular CTGF upregulation is mainly seen in podocytes and in some parietal epithelial cells. G, H CTGF is strongly expressed in periglomerular fibrotic lesions, and in the media of arcuate arteries of group V (1K32W). Endothelial cells of arteries are negative for CTGF, and CTGF is not detected in arterioles. Long arrows = Segmental adhesion and scar; short arrows = interstitial fibroblasts; open arrows = arterioles; open arrowheads = parietal epithelium; black arrowheads = visceral epithelium (podocytes); Latin cross = flattened tubular epithelium; single asterisk = internal elastic lamina; double asterisk = external elastic lamina.
Histomorphological Changes

At 32 weeks, UNX-SHR showed focal segmental glomerulosclerosis (FSGS) associated with tubular atrophy and interstitial fibrosis (fig. 4, 5, 6). The mean percentage of sclerotic glomeruli was 10.27 ± 4.48%, which is significantly higher than that in any of the other groups (p < 0.01), while there was no significant difference in number of FSGS lesions between the other groups (fig. 4G). Arcuate arteries and interlobar arteries showed hypertrophy of the media 32 weeks after UNX, but there was neither fibrous intimal thickening nor hyalinosis.

CTGF Expression Level in UNX

CTGF mRNA and protein expression in renal sections from control SHR was weakly to moderately intense and mainly confined to glomerular podocytes, as shown by ISH and IHC. In contrast, 32 weeks after UNX, CTGF mRNA was focally overexpressed, especially in the sclerosing glomeruli. The mean surface area of positive staining for CTGF mRNA was similar in all groups, except in group V (1K32W). At 32 weeks after UNX, mean glomerular CTGF expression was increased 1.3-fold over baseline, and sclerosing glomeruli in group V had 1.7-fold higher expression than normal appearing glomeruli of the same group (fig. 4G). Glomerular CTGF protein staining by IHC was congruent with ISH for CTGF mRNA, with predominant localization in podocytes and parietal epithelial cells, and in the periglomerular area (fig. 4, 5).

In control rat kidneys of group I (2K0W), tubulointerstitial CTGF and α-SMA were barely detectable. Until 17 weeks after UNX, tubulointerstitial CTGF mRNA, CTGF protein, and α-SMA expression were not significantly different in group III (1K17W) as compared to group II (2K32W). At 32 weeks after UNX, CTGF mRNA, CTGF protein, and α-SMA expression were upregulated in UNX-SHR (group V, 1K32W) as compared to sham-operated SHR (group IV, 2K32W). Strong CTGF mRNA and protein expression was detected in tubulointerstitial fibrotic areas, mainly in fibroblasts, with a 5-fold increase for CTGF mRNA in group V (1K32W) as compared to group IV (2K32W; p < 0.001; fig. 6E). CTGF was prominent in periglomerular cells, with concomitant expression by the parietal epithelial cells, although fibrosis was only mild. CTGF was expressed also in some flattened to spindle-shaped tubular epithelial cells. The induction of CTGF in the tubulointerstitial compartment during the later phase of renal injury of UNX-SHR was strongly associated with tubulointerstitial fibrosis and α-SMA accumulation. In contrast, CTGF mRNA and protein were not expressed by infiltrating mononuclear cells (fig. 4, 5, 6).

In control rats of group I (2K0W), arterial CTGF mRNA expression was limited to the adventitia of arcuate arteries, while CTGF protein was weakly positive in the media. Strong expression of CTGF mRNA and protein in the media of arcuate and interlobar arteries developed over time and was most pronounced in group V (1K32W). In all groups, CTGF protein and mRNA expression was consistently higher in arcuate and interlobar arteries than in interlobular arteries. In all arteries, CTGF mRNA tended to be higher in groups IV (2K32W) and V (1K32W) than in control rats (group I, 2K0W). CTGF mRNA and protein were not detected in the arterioles in any of the groups (fig. 7).
Correlation of Urinary and Plasma CTGF with Histopathology

Plasma and urinary CTGF correlated significantly with the percentage of FSGS lesions, with glomerular and tubulointerstitial CTGF expression, and with glomerular and tubulointerstitial α-SMA expression. Glomerular and tubulointerstitial CTGF expression correlated with α-SMA expression in the glomerulus and in the tubulointerstitial area, respectively. CTGF expression in the large (arcuate and interlobar) arteries correlated significantly with plasma, glomerular, and tubulointerstitial CTGF, but not with functional and histological parameters (table 3).

Fig. 6. CTGF mRNA in tubulointerstitial area. CTGF mRNA was assessed by in situ hybridization. A, B CTGF mRNA is increasingly expressed in the fibrotic tubulointerstitial area in UNX-SHR at 32 weeks (group V; 1K32W). PAS stain shows that in severe tubulointerstitial lesions, most of the infiltrating cells do not express CTGF mRNA. Some flattened tubular epithelial cells expressed CTGF mRNA (arrow). C, D Azan stain shows that in the focal fibrotic area CTGF mRNA is also expressed by flattened tubular epithelial cells (arrow) and by interstitial cells (arrowhead). E Morphometric analysis of ISH for CTGF mRNA expression and IHC for α-SMA in the tubulointerstitial area showed that both were significantly more elevated at 32 weeks post UNX (group V; 1K32W) than in any of the other groups. ## p < 0.005 versus I, II, III and IV.
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Fig. 7. CTGF mRNA in the arcuate artery. CTGF mRNA was assessed by in situ hybridization. A In control SHR (group I; 2K0W), CTGF is only detected in the adventitia of the arcuate artery. I.E.L. = Internal elastic lamina; E.E.L. = external elastic lamina. B, C CTGF is strongly expressed in the media of the arcuate artery in group IV (2K32W; B) and group V (1K32W; C). d Morphometric analysis shows that CTGF expression in arcuate and interlobar arteries is significantly higher in group V (1K32W) than in any other group. CTGF expression in the interlobular arteries in groups IV (2K32W) and V is prone to be higher than that in the other 3 groups. No CTGF mRNA expression was detected in arterioles in any group. ** p < 0.05 versus I, II, and III; ## p < 0.01 versus I, II, III, and IV.

Table 3. Correlation (R values) between proteinuria, plasma and urinary CTGF, and pathological parameters in the SHR model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CTGF plasma</th>
<th>CTGF urine</th>
<th>FSGS</th>
<th>CTGF glomeruli</th>
<th>α-SMA glomeruli</th>
<th>CTGF TI</th>
<th>α-SMA TI</th>
<th>CTGF large art</th>
<th>CTGF small art</th>
<th>Proteinuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTGF plasma</td>
<td>0.729 b</td>
<td>0.711 c</td>
<td>0.584 a</td>
<td>0.840 c</td>
<td>0.902 d</td>
<td>0.927 d</td>
<td>0.697 b</td>
<td>0.106</td>
<td>0.831 c</td>
<td></td>
</tr>
<tr>
<td>CTGF urine</td>
<td>0.873 d</td>
<td>0.618 a</td>
<td>0.639 a</td>
<td>0.670 a</td>
<td>0.898 c</td>
<td>0.543</td>
<td>0.235</td>
<td></td>
<td>0.774 c</td>
<td></td>
</tr>
<tr>
<td>CTGF glomeruli</td>
<td>0.799 b</td>
<td>0.685 b</td>
<td>0.586</td>
<td>0.750 c</td>
<td>0.352</td>
<td>0.500</td>
<td></td>
<td>0.674 b</td>
<td></td>
<td>0.022</td>
</tr>
<tr>
<td>CTGF TI</td>
<td></td>
<td></td>
<td>0.828 c</td>
<td>0.733 c</td>
<td>0.132</td>
<td>0.798 c</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>CTGF large art</td>
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</table>

TI = Tubulointerstitium; art = artery
a p < 0.05; b p < 0.01; c p < 0.005; d p < 0.001.
Immunohistochemistry for TGF-β1 in UNX-SHR Kidney
TGF-β was barely detectable in glomeruli throughout the experiment. In contrast, TGF-β was strongly upregulated in the tubulointerstitial area, including expression in some flattened tubular epithelial cells 32 weeks after UNX. Like CTGF, TGF-β was prominent in the media of the arcuate and interlobar arteries, but no staining was observed in arterioles and small interlobular arteries (fig. 8).

Discussion
This study has revealed that an increase in soluble and tissue CTGF is both a marker and a likely key factor in progression of human and experimental hypertensive renal and vascular disease.
In patients with hypertensive nephrosclerosis, we detected higher CTGF in plasma, urine, and glomerular podocytes than in control subjects and in patients with hypertension stage 1. Accordingly, in UNX-SHR, we found increased plasma, urinary, and tissue expression of CTGF in association with glomerulosclerosis and tubulointerstitial fibrosis, and with arterial media hypertrophy. These data are an important extension of previous studies in SHR, which showed that renin-angiotensin-aldosterone system blockade inhibited upregulation of CTGF expression in kidneys of SHR (2K) without glomerulosclerosis [11, 12].
In UNX-SHR, we detected sclerotic changes in 10% of the glomeruli 32 weeks after UNX. At this time point, mean glomerular CTGF expression was increased, mainly due to a strong increase in CTGF in glomeruli undergoing sclerosis. These findings suggest that hyperfiltration and subsequent focal segmental sclerosis rather than systemic hypertension per se are important elements in the upregulation of CTGF. The pattern of CTGF expression in the sclerosing glomeruli after UNX was similar to that
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observed in human hypertensive glomerulosclerosis, and also in our previous studies of experimental and human diabetic nephropathy [18, 19, 21]. Interestingly, an increase in CTGF expression in glomeruli was not accompanied by a concomitant increase in glomerular TGF-β expression. This indicates that CTGF overexpression in podocytes is independent of TGF-β and may be related to, e.g. stretch forces resulting from glomerular hypertension, analogous to in vitro observations on stretch- and other cytoskeleton-mediated induction pathways regulating CTGF expression in mesangial cells [22].

We have previously proposed a role of CTGF in the interaction between parietal epithelial cells and periglomerular myofibroblasts based upon observations in Thy1.1 nephritis and in human renal biopsies [10, 20]. Also in the present study, we found concomitant expression of α-SMA and CTGF in the periglomerular cells and of CTGF in adjacent parietal epithelial cells of UNX-SHR at 32 weeks. These findings support our notion that paracrine stimulation by CTGF from parietal epithelial cells may induce α-SMA-positive myofibroblasts and fibrosis around glomeruli with segmental lesions. Of note, periglomerular fibrosis in IgA nephropathy correlates with prognosis, but for hypertensive nephrosclerosis this has not been studied thus far [23].

Clinical disease progression correlates even better with interstitial changes than with glomerular changes [24]. Many potential fibrogenic factors, including TGF-β, have been implicated in tubulointerstitial injury [3, 25]. Previously, we reported that in human renal biopsies CTGF mRNA correlated with the degree of tubulointerstitial damage [10]. In the present study, CTGF was strongly induced in the tubulointerstitial compartment during the late phase of renal injury in hypertensive rats, in association with tubulointerstitial fibrosis, α-SMA accumulation, and TGF-β expression. The congruent staining patterns suggest that at least part of the induction of tubulointerstitial CTGF might be secondary to increased TGF-β expression.

The overall picture suggests that CTGF produced by myofibroblasts plays an important role in the development of chronic tubulointerstitial fibrosis, possibly by autocrine and paracrine stimulation.

Emerging evidence indicates that a subset of interstitial fibroblasts has actually originated from tubular epithelial cells via epithelial-to-mesenchymal transition [26] and that CTGF may be critically involved in this process [27, 28]. In line with this, we observed that in UNX-SHR, CTGF mRNA and protein were expressed in occasional flattened spindle-shaped epithelial cells lining atrophic tubules, which might be undergoing epithelial-to-mesenchymal transition.

In hypertensive rats, hypertrophy of the aorta and other large vessels is generally more pronounced than hyperplasia and remodeling [29, 30]. Recently, it was shown that systemic renin-angiotensin-aldosterone system blockade diminished CTGF expression in the aorta of SHR (2K) [11, 12]. In the present study, we detected upregulation of CTGF mRNA and protein in the hypertrophic media of arcuate and interlobar arteries of SHR 32 weeks after UNX. These findings suggest that CTGF might be significantly involved
CTGF in hypertensive nephrosclerosis

also in hypertension-induced hypertrophy of large arteries of the kidney. In contrast to medial hypertrophy of the large arteries, prominent remodeling and hyperplasia rather than hypertrophy are present in arterioles and smaller arteries in SHR and in humans with essential hypertension [31]. Of interest, we detected no CTGF mRNA and protein, nor TGF-β protein expression in arterioles, and only weak expression in the interlobular arteries. It thus appears that overexpression of CTGF is linked to medial hypertrophy in large arteries rather than to remodeling and hyperplasia of smaller arteries and arterioles.

In summary, our data support the notion that tissue, urinary, and plasma CTGF are valuable biomarkers of progression of human hypertensive nephrosclerosis. In addition, local overexpression of CTGF is probably a key factor in hypertensive vascular injury and renal fibrosis.

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