COMP–Angiopoietin-1 Ameliorates Renal Fibrosis in a Unilateral Ureteral Obstruction Model

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Injury to the renal microvasculature may be a major factor in the progression of renal disease; therefore, protection of endothelial cells (EC) in renal vasculature may have a therapeutic role in renal fibrosis. Recently, a soluble, stable, and potent angiopoietin-1 (Ang1) variant, cartilage oligomeric matrix protein (COMP)-Ang1, was developed. The contribution of COMP-Ang1 in renal interstitial fibrosis, however, remains to be clarified. This study investigated the effects of COMP-Ang1 on peritubular capillary EC in the renal cortex and the renal fibrogenic process that is triggered by unilateral ureteral obstruction. COMP-Ang1 preserved renal platelet-EC adhesion molecule-1– and Tie2-positive EC. Morphologic examination indicated less tubular injury and tubulointerstitial fibrosis in mice that received COMP-Ang1 than vehicle-treated mice. Interstitial type I collagen and myofibroblast accumulation were significantly suppressed by COMP-Ang1 treatment. COMP-Ang1 increased Tie2 and Akt phosphorylation in ureteral obstructed kidneys. Renal surface microvasculature and renal blood flow were higher after treatment with COMP-Ang1 than with vehicle. COMP-Ang1 treatment decreased monocyte/macrophage infiltration, tissue levels of TGF-β1, and Smad 2/3 phosphorylation and increased Smad 7 in the obstructed kidney. These results demonstrate that COMP-Ang1 treatment can decrease the progression of renal fibrosis in unilateral ureteral obstruction. COMP-Ang1 may be an endothelium-specific therapeutic modality in fibrotic renal disease.

M ost forms of chronic renal disease progress to tubulointerstitial fibrosis. The severity of tubulointerstitial changes is the best indicator of the progression of renal dysfunction (1,2). Renal tubulointerstitial fibrosis is a common feature in unilateral ureteral obstruction (UUO). In humans, chronic and acute ureteral obstruction can occur in various clinical situations.

Injury to the renal microvasculature is a major factor that contributes to the progression of renal disease (3). In particular, injury to the peritubular capillary network of the kidney is a key factor in tubulointerstitial disease (4,5). Impaired angiogenesis may occur in the diseased kidney and can contribute to renal scarring (6). Renal ischemia that is caused by vascular obliteration is a major contributor to renal scarring (7). Ohashi et al. (8) suggested that peritubular capillary regression may contribute to tubulointerstitial scarring in the UUO model. Therefore, endothelial cells (EC) play an important role in renal disease progression in the UUO kidney. Because renal microvasculature injury constitutes an important mechanism in renal fibrosis, a growth factor or cytokine with an endothelial protective or angiogenic effect may ameliorate renal fibrosis in the UUO model (9–11).

Angiopoietin-1 (Ang1) is a widely expressed ligand for the Tie2 tyrosine kinase receptor that is expressed on EC (12), and it regulates vascular growth, development, maturation, and permeability (13,14). Ang1 has potential therapeutic applications in inducing angiogenesis, enhancing EC survival, and preventing vascular leakage. We have demonstrated that Ang1 increases endothelial survival and has an anti-inflammatory effect (15,16). We recently developed a soluble and potent Ang1 variant, cartilage oligomeric matrix protein (COMP)-Ang1, which is more potent than native Ang1 in phosphorylating Tie2 and signaling via Akt in primary cultured EC (17). COMP-Ang1 rescues radiation-induced apoptosis in microcapillary EC of the intestinal villi (18). Recently, we also demonstrated that sustained COMP-Ang1 treatment can produce long-lasting tracheal vascular enlargement and increase blood flow (19). In kidney development, Ang1 and Tie2 play roles in the maturation of glomeruli and the vasa rectae (20). In addition, Ang1 expression is increased in folic acid–induced nephrotoxicity

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(21). Therapeutic application of Ang1 in kidney disease has not been studied.

We examined whether COMP-Ang1 preserves renal EC, has an anti-inflammatory effect, or indirectly suppresses renal fibrotic processes that are triggered by UUO. We demonstrate that COMP-Ang1 treatment preserves renal EC, decreases F4/80-positive cell infiltration, and decreases renal fibrosis in the UUO model. These findings indicate that COMP-Ang1 may have therapeutic applications in the treatment of EC injury and renal fibrosis.

Materials and Methods

Animal Experiments: UUO Model

Animal studies were reviewed and approved by the Institutional Animal Care and Use Committee of Chonbuk National University Medical School. Male Tie2–green fluorescence protein (GFP) transgenic mice (FVB/N; Jackson Laboratory, Bar Harbor, ME; 20 to 30 g body wt) were used for platelet-EC adhesion molecule-1 (PECAM-1) immunostaining, and male C57BL/6 mice (Charles River Korea, Seoul, Korea; 20 to 30 g body wt) were used in other experiments (22). COMP-Ang1 and recombinant adenosine–expressing COMP-Ang1, LacZ, and sTie2-Fc were constructed using previously published methods (19). For adenoviral treatment, Ade-COMP-Ang1 and sTie2-Fc or Ade-LacZ (vehicle) diluted in 50 l of sterile 0.9% NaCl was injected intravenously through the tail vein. In preliminary experiments, circulating serum levels of COMP-Ang1 increased at 3 d after treatment, peaked at 5 d, and declined thereafter. To evaluate the effect of high levels of COMP-Ang1 in the UUO model, mice received an intravenous injection of COMP-Ang1 adenovirus or vehicle adenovirus 3 d before and 2 wk after UUO. An incision was made in the midline of the abdomen, and the left proximal ureter was exposed and was ligated at two separate locations using 3-0 silk.

Measurement of Systemic Mean Arterial Pressure, Peak Renal Artery Blood Velocity, and Renal Cortical Blood Flow

Cannulas were placed into the carotid artery (PE10) to measure systemic mean arterial pressure and in the jugular vein (PE10) for infusion of 0.9% NaCl solution (1.5 ml/min/g body wt). Peak renal artery blood velocity was measured with a 10- to 15-MHz linear transducer (ACUSON Sequoia C512; Siemens, Malvern, PA) (23). Changes in the blood flow in the superficial renal cortex were measured using a laser-Doppler flow probe (1.2-mm diameter, Type N; Transonic Systems, Ithaca, NY), as described previously (24).

Immunoblotting

Immunoblotting was performed as described (25). Primary antibodies to Tie2, phospho-Tie2, Akt, phospho-Akt, actin, phospho-Smad 2/3, and Smad 7 were purchased from Cell Signaling Technology Inc. (Beverly, MA). Signals were analyzed by densitometric scanning (LAS-1000; Fuji Film, Tokyo, Japan).

Histology and Immunohistochemistry

Sections of sham-operated, contralateral, and UUO kidneys were fixed in 10% neutral buffered formalin and embedded in paraffin. Tissue was sectioned at 5 mm and stained with periodic acid-Schiff for light microscope analysis. The presence of interstitial fibrosis was assessed in slides that were stained with Masson’s trichrome. Immunostaining was performed as described previously (25). Anti-mouse type I collagen (SouthernBiotech, Birmingham, AL), anti-α-smooth muscle actin (α-SMA; DakoCytomation, Glostrup, Denmark), anti-PECAM-1 (Chemicon International, Temecula, CA), and anti-mouse F4/80 (Srotec, Oxford, UK) were used for frozen sections. Digital images of GFP and PECAM-1 were obtained with a Zeiss Apotome microscope and a Zeiss LSM 510 confocal microscope (Carl Zeiss, Göttingen, Germany). The positive area of PECAM-1, type I collagen, and α-SMA was evaluated from the unit area and expressed as a percentage per unit area using a digital image analysis program (AnalySIS; Soft Imaging System, Münster, Germany).

Histopathologic Scoring

Histopathologic scoring was made in the cortex using a blind method. Tubular injury was assessed by grading tubular dilation, epithelial desquamation, and brush border loss in 10 randomly chosen, nonoverlapping fields (×200 magnification) using methods previously described (26).

Total Collagen Assay

Hydroxyproline concentrations in hydrolyzed (6 M HCl, 110°C, 12 h) frozen kidney samples were measured (26,27).

Contrast-Enhanced In Vivo Microrthy and Stereologic Analyses

To obtain high-contrast views of organ microcirculation, we modified previously reported methods (28). In brief, high-molecular-weight (500,000) dextran labeled with FITC (Sigma-Aldrich, St. Louis, MO) was administered intravenously (0.05 ml, 25 mg/ml). Kidneys were exposed by retroperitoneal incision and episcopically illuminated (FITC-dextran; excitation = 490 nm, emission = 520 nm) under a fluorescence intravital microscope. Real-time imaging was recorded at 30 frames/s using the Cascade 650 CCD camera (Roper Scientific, Tucson, AZ) assisted with MetaMorph software (Universal Imaging Corp., Sunnyvale, CA).

Quantitative stereology was used to determine the vascular volume fraction and branch-point density of the kidney microvasculature at 2 and 4 wk after UUO as described previously (28). Glomeruli were omitted. Functional microvessel density and vessel segment length were measured as described previously (29). Ten randomly selected regions (500 ×300 μm) were evaluated at each time point.

ELISA of COMP-Ang1 and TGF-β1

Serum concentrations of COMP-Ang1 were determined as described previously (19). Levels of TGF-β1 were measured at 0, 5, 7, and 14 d after UUO and after treatment with COMP-Ang1 adenovirus.

Determination of Blood and Urine Parameters

Urinary protein, urinary creatinine, and blood urea nitrogen levels were determined using an autoanalyzer (HITACHI clinical analyzer 7180; Hitachi High-Technologies Co., Tokyo, Japan).

Statistical Analyses

Data are expressed as mean ± SD. The t test was used to compare the means of normally distributed continuous variables. P < 0.05 indicated statistical significance.

Further details regarding the experimental protocols, materials used, and experimental methods can be found in the data supplement Expanded Materials and Methods Section.
Results

COMP-Ang1 Preserves Renal PECAM-1– and Tie2-Positive EC in UUO

Serum levels of COMP-Ang1 were 0 μg/ml at 0 d, 2.8 ± 0.5 μg/ml at 1 d, 3.9 ± 0.7 μg/ml at 3 d, 4.3 ± 0.6 μg/ml at 5 d, 3.5 ± 0.6 μg/ml at 7 d, 1.7 ± 0.2 μg/ml at 14 d, and 0.2 ± 0.3 μg/ml at 28 d (mean ± SD; n = 5 in each group). Because COMP-Ang1 is a potent angiogenic factor and a ligand for Tie2 expressed on EC, we asked whether COMP-Ang1 preserves the renal PECAM-1– and Tie2-positive EC in UUO. Tie2-GFP transgenic mice were treated with COMP-Ang1 or vehicle. After 2 wk, the PECAM-1–positive peritubular EC in vehicle-treated UUO kidneys were slightly increased compared with sham-operated kidneys that were treated with vehicle, but Tie2 in peritubular capillaries in vehicle-treated UUO kidneys was not changed. COMP-Ang1 increased the expression of PECAM-1 and Tie2 in peritubular capillaries compared with vehicle-treated UUO kidneys (Figure 1A). After 4 wk, PECAM-1– and Tie2-positive EC expression was decreased in vehicle-treated UUO kidneys compared with sham-operated kidneys (Figure 1B). However, the expression of PECAM-1–positive EC and Tie2 in peritubular capillaries was increased in COMP-Ang1–treated UUO kidneys compared with vehicle-treated UUO kidneys (Figure 1). In vehicle-treated mice, PECAM-1 and Tie2 expression in contralateral kidneys was not changed compared with sham-operated kidneys at 2 and 4 wk (Figure 1). In contralateral and sham-operated kidneys, COMP-Ang1 slightly increased the expression of PECAM-1 and Tie2 in COMP-Ang1–treated kidneys compared with vehicle-treated kidneys at 2 and 4 wk. In glomeruli, the expression of PECAM-1–positive EC and Tie2 was decreased in UUO compared with sham-operated kidneys but increased in COMP-Ang1–treated compared with vehicle-treated kidneys (Figure 1). Area densities of PECAM-1 expression in a given microscopic field area (0.22 mm²) for sham-operated kidney, contralateral kidney, and UUO kidneys were 3.8 ± 0.2, 3.6 ± 0.2, and 4.3 ± 0.2% (mean ± SD from 15 microscopic fields), respectively, after vehicle treatment (at 2 wk); 7.9 ± 0.2, 7.8 ± 0.3, and 10.5 ± 0.9%, respectively, after COMP-Ang1 treatment (at 2 wk); 3.3 ± 0.2, 3.3 ± 0.3, and 0.5 ± 0.1%, respectively, after vehicle treatment (at 4 wk); and 7.6 ± 0.3, 7.1 ± 0.2, and 2.3 ± 0.3%, respectively, after COMP-Ang1 treatment (at 4 wk; Table 1).

COMP-Ang1 Confers Tubulointerstitial Morphologic Protection against UUO

Two weeks after UUO, obstructed kidneys of vehicle-treated mice showed destruction of the renal tubules with significant mononuclear inflammatory infiltration and mild stromal fibrosis (Figure 2, B and C). COMP-Ang1–treated UUO kidneys showed relatively well-preserved renal tubular structure and less inflammatory cell infiltration. Stromal fibrosis in COMP-Ang1–treated kidneys also was milder than that in the vehicle-treated kidneys (Figure 2B). Four weeks after UUO, obstructed kidneys of vehicle-treated mice were enlarged, and dilated pelvises and calyces and a thin rim of remaining cortex were seen, whereas substantial renal parenchyma was preserved in UUO kidneys of COMP-Ang1–treated mice (Figure 2). Histologically, vehicle-treated UUO kidneys showed more destructive changes (Figure 2). Structurally preserved renal tubules were not easily recognized and showed severe stromal fibrosis. Stromal inflammatory cell infiltration was decreased according to the progression of fibrosis in obstructed kidneys. COMP-Ang1–treated UUO kidneys showed more preserved renal tubular structure and milder fibrosis, similar to that of vehicle-treated kidneys 2 wk after UUO. Sham-operated and contralateral kidneys showed no significant morphologic change after COMP-Ang1 treatment compared with vehicle-treated kidneys. Previous exposure of mice to COMP-Ang1 resulted in protection against tubulointerstitial histologic damage of approximately 38 and 24% at 2 and 4 wk after UUO, respectively (Figure 2, B and C).

Renal Fibrosis Induced by UUO Is Ameliorated after COMP-Ang1 Treatment

To determine the effect of COMP-Ang1 on interstitial fibrosis, we stained slides with Masson's trichrome and measured blue-stained collagen deposition. Collagen deposition in obstructed kidneys increased 2 and 4 wk after UUO (Figure 3, A...
and B). Fibrosis was not observed in sham-operated and contralateral kidneys. Increased collagen deposition at 2 and 4 wk after UUO was significantly decreased in COMP-Ang1–treated kidneys by 29 and 30%, respectively, compared with vehicle-treated UUO kidneys (Figure 3B). Type I collagen was deposited in vehicle-treated UUO kidneys compared with sham-operated kidneys 2 and 4 wk after surgery (Figure 3, C and D). COMP-Ang1 significantly decreased interstitial type I collagen expression in UUO kidneys by approximately 64 and 37% at 2 and 4 wk after UUO, respectively (Figure 3D).

/H9251-SMA is a marker of myofibroblast differentiation in response to obstruction. After 1 and 2 wk, COMP-Ang1 significantly decreased /H9251-SMA expression by approximately 37 and 31% compared with the vehicle-treated UUO kidneys, respectively (Figure 3, E and F). Hydroxyproline content, a measure of total collagen, increased 3.5- and 6.5-fold in the vehicle-treated UUO kidneys compared with sham-operated kidneys 2 and 4 wk after surgery, respectively; COMP-Ang1 decreased the UUO-induced increased hydroxyproline content by 27 and 38%, respectively (Figure 3G). The data indicate that COMP-Ang1 ameliorates UUO-induced renal fibrosis.

COMP-Ang1 Increases Renal Tie2 and the Phosphorylation of Tie2 and Akt in UUO

COMP-Ang1 acts on endothelial receptor Tie2 and phosphorylates Tie2 (30). In Western blot analyses, COMP-Ang1 increased Tie2 and Tie2 phosphorylation at 5, 7, and 14 d after UUO compared with vehicle (Figure 4A). The mean increases in Tie2 and Tie2 phosphorylation after COMP-Ang1 treatment were 1.3- and 1.25-fold, respectively, at 5 d, indicating that treatment increases kidney endothelial Tie2 and Tie2 phosphorylation.

To determine the involvement of Tie2 in COMP-Ang1–induced Tie2 phosphorylation in UUO kidney, we pretreated mice with sTie2-Fc at 24 h before COMP-Ang1 treatment. Pretreatment reversed COMP-Ang1–induced Tie2 phosphorylation 5 d after surgery (Figure 4B), indicating that COMP-Ang1–induced Tie2 phosphorylation occurs primarily through Tie2 activation in kidney.

Because phosphorylation of Akt in EC is an important pathway downstream of COMP-Ang1 (18), we assayed Akt phosphorylation in COMP-Ang1–treated UUO kidneys. COMP-Ang1 increased Akt phosphorylation at 5, 7, and 14 d after

| Table 1.

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aData are mean ± SD. COMP-Ang1, cartilage oligomeric matrix protein-angiopoietin-1; CON, contralateral kidney; PECAM-1, platelet-endothelial cell adhesion molecule-1; Sham, sham-operated mice; UUO, unilateral ureteral obstruction. 

bP < 0.05 versus sham-operated kidneys on the same day in vehicle treatment group.

cP < 0.05 versus vehicle-treated kidneys on the same day in each group.

dP < 0.05 versus sham-operated kidneys on the same day in COMP-Ang1 treatment group.

Figure 2. COMP-Ang1 reduces renal tubulointerstitial damage after UUO. (A and B) Representative pictures of tubulointerstitial lesions: At 4 wk at low magnification (A) and at 2 and 4 wk after UUO and sham surgery at high magnification (PAS stain; B). Mice were treated with 1 × 10⁹ pfu Ade-COMP-Ang1. Tubular casts, severe cell infiltration, tubular atrophy, and interstitial fibrosis were observed 2 and 4 wk after UUO in vehicle-treated mice. In contrast, such lesions were significantly reduced in COMP-Ang1-treated mice. Bar = 50 μm. (C) Semi-quantitative scoring of tubular injury revealed more damage in vehicle-treated than in COMP-Ang1–treated kidneys 2 and 4 wk after UUO (n = 8 for each experimental group). Data are expressed as means ± SD. Gray bars indicate vehicle treatment; red bars indicate COMP-Ang1 treatment. *P < 0.05 versus sham-operated mice treated with vehicle; #P < 0.05 versus UUO kidneys treated with vehicle on the same day; §P < 0.05 versus sham-operated mice treated with COMP-Ang1.
UUO compared with vehicle (Figure 4C). The mean increases in Akt phosphorylation were 1.7-, 1.4-, and 1.3-fold at 5, 7, and 14 d, respectively.

Figure 3. COMP-Ang1 reduces renal interstitial fibrosis, type I collagen deposition, fibroblast activation, and α-SMA expression in UUO kidneys. (A) Light-field photomicrographs of Masson’s trichrome–stained sections of sham-operated and UUO kidneys treated with vehicle or COMP-Ang1 at 4 wk after surgery. (B) Semiquantitative score of tubulointerstitial fibrosis in Masson’s trichrome–stained sections. Seven randomly selected high-power fields were quantified and averaged to obtain the value for each mouse (n = 6 for each experimental group). (C) Immunohistochemical study for type I collagen from sham-operated and UUO kidneys treated with vehicle or COMP-Ang1 at 4 wk after surgery. Bar graph shows the ratio of the positively stained area to the total field (n = 6 for each experimental group). (D) Immunohistochemical study for α-SMA from sham-operated and UUO kidneys treated with vehicle or COMP-Ang1 at 2 wk after surgery. The periarterial area is strongly stained with α-SMA (arrows). (E) Semiquantitative score of tubulointerstitial α-SMA–positive area in the sham-operated and UUO kidneys from 1 and 2 wk after surgery (n = 6 for each experimental group). (F) Hydroxyproline content was determined by a colorimetric assay after acid hydrolysis and expressed as a percentage of dry tissue weight (n = 6 for each experimental group). Bar = 50 μm. Data are expressed as mean ± SD. Gray bars indicate vehicle treatment; red bars indicate COMP-Ang1 treatment. *P < 0.05 versus sham-operated mice treated with vehicle; †P < 0.05 versus UUO kidneys treated with vehicle on the same day; ‡P < 0.05 versus UUO kidneys treated with vehicle on the same day; §P < 0.05 versus UUO kidneys treated with COMP-Ang1 on the same day.

Figure 4. COMP-Ang1 increases Tie2 and phosphorylation of Tie2 and Akt in UUO kidneys. (A) Immunoblotting of phospho-Tie2 in sham-operated or UUO kidneys treated with COMP-Ang1 or vehicle. Each lane contains 150 μg of total protein. Blots were probed with anti–phospho-Tie2 antibody. The membrane was stripped and reprobed with anti-actin antibody to verify equal loading of protein in each lane. Densitometric analyses are presented as the relative ratio of Tie2 to actin (□) and phospho-Tie2 to Tie2 (■). The relative ratio measured in sham-operated kidneys is arbitrarily presented as 1. Results were similar from six independent experiments. (B) Immunoblotting of phospho-Tie2 in UUO kidneys treated with COMP-Ang1 with or without sTie2-Fc at 5 d after surgery. Mice were pretreated with 1 × 10⁹ pfu Ade-sTie2-Fc 24 h before treatment with 1 × 10⁹ pfu Ade-COMP-Ang1. Each lane contains 150 μg of total protein. Densitometric analyses are presented as in A. Results were similar from six independent experiments. (C) Immunoblot analyses of Akt and phospho-Akt in sham-operated and UUO kidneys treated with COMP-Ang1 or vehicle. Each lane contains 100 μg of total protein. Blots were probed with anti–phospho-Akt antibody. The membrane was stripped and reprobed with anti-Akt antibody to verify equal loading of protein in each lane. Densitometric analyses are presented as the relative ratio of phospho-Akt to Akt. The relative ratio measured in sham-operated kidneys is arbitrarily presented as 1. Data are expressed as means ± SD. Results were similar from six independent experiments. *P < 0.05 versus UUO kidneys treated with vehicle on the same day; †P < 0.05 versus UUO kidneys treated with vehicle on the same day; ‡P < 0.05 versus UUO kidneys treated with COMP-Ang1 on the same day.
COMP-Ang1 Preserves Renal Microvasculature in In Vivo Vital Microscopy

Compared with sham-operated kidneys, decreased surface microvasculature in UUO kidneys commonly was observed after treatment with COMP-Ang1 or vehicle, but some remnant vessels were abnormally dilated and tortuous in vehicle-treated UUO kidneys. COMP-Ang1 lessened the UUO-induced decrease of surface microvasculature and ameliorated abnormally dilated and tortuous vessels compared with vehicle-treated UUO kidneys (Figure 5A; supplementary videos). With the use of Figure 5A as a map of functional microvessels, vascular volume fraction, functional microvessel density, and branch-

![Figure 5A](image)

**Figure 5.** Effect of adenoviral COMP-Ang1 on microcirculation in kidney after UUO. (A) Representative views of kidney microcirculation after treatment with COMP-Ang1 or vehicle treatment. Note that COMP-Ang1 lessened the UUO-induced decrease of surface microvasculature and ameliorated abnormally dilated and tortuous vessels (arrows) in UUO kidneys compared with vehicle. All images were acquired using eight microscopic fields and represent a single frame from an in vivo vital microscopy sequence in COMP-Ang1– or vehicle-treated kidneys at 2 and 4 wk after UUO. Bar = 100 μm. (B through E) Stereologic assessment of microvascular features. Values are from analysis of single-frame images of kidney surface microvasculature after treatment with COMP-Ang1 or vehicle. In UUO kidneys, vascular volume fraction (B) and functional microvessel density (C) were significantly reduced, but because of the greater reduction in vessel branch-point density (D), vessel segment length (E) was increased relative to sham-operated kidneys. However, COMP-Ang1 significantly increased vascular volume fraction (B) and functional microvessel density (C) in UUO kidneys compared with vehicle. Because COMP-Ang1 increased the vessel branch-point density (D) in UUO kidneys, vessel segment length decreased after COMP-Ang1 treatment. Data are expressed as mean ± SD. Gray bars indicate vehicle treatment; red bars indicate COMP-Ang1 treatment (n = 4 for each experimental group). *P < 0.05 versus sham-operated mice treated with vehicle; †P < 0.05 versus UUO kidneys treated with vehicle on the same day; ‡P < 0.05 versus sham-operated mice treated with COMP-Ang1. Magnification, ×100 in A.
point density at 2 and 4 wk were decreased in vehicle-treated UUO kidneys compared with sham-operated kidneys (reductions of 47, 39, and 57% at 2 wk, respectively; reduction of 54, 48, and 65% at 4 wk, respectively; Figure 5, B through D). As branch-point density decreased in vehicle-treated UUO kidneys, vessel segment length increased 180 and 225% relative to sham-operated kidneys at 2 and 4 wk, respectively (Figure 5E). However, vascular volume fraction, functional microvessel density, and branch-point density of COMP-Ang1–treated UUO kidneys at 2 and 4 wk were significantly greater than those of vehicle-treated UUO kidneys (increases of 140, 135, and 184% at 2 wk, respectively; increases of 153, 132, and 185 at 4 wk, respectively; Figure 5, B through D). COMP-Ang1 decreased segment length (23 and 16% at 2 and 4 wk, respectively) compared with vehicle-treated UUO kidneys (Figure 5E). These results indicate that COMP-Ang1 reduces UUO-induced decrease in vascular volume, length, and branch-point density and reduces the UUO-induced increase in vessel segment length.

COMP-Ang1 Increases Renal Cortical Blood Flow and Peak Renal Artery Blood Velocity in UUO Mice

The influence of COMP-Ang1 on systemic arterial BP, renal cortical blood flow, and peak renal artery blood velocity in UUO mice is illustrated in Figure 6. Compared with sham-operated mice, systemic mean arterial pressure of UUO mice was not significantly different after vehicle or COMP-Ang1 treatment (Figure 6A), but renal cortical blood flow was significantly decreased by 65 and 92% at 2 and 4 wk, respectively, with vehicle treatment (Figure 6B). After COMP-Ang1 treatment, renal cortical blood flow was significantly increased 1.6- and 2.9-fold at 2 and 4 wk, respectively, compared with vehicle treatment (Figure 6B). Two weeks after UUO, peak renal artery blood velocity after vehicle treatment was not significantly changed compared with sham-operated mice. Although peak renal artery blood velocity in COMP-Ang1–treated kidneys was increased 1.2-fold at 2 wk, compared with sham-operated mice, peak renal artery blood velocity was not significantly changed at 2 wk, compared with vehicle-treated mice. However, peak renal artery blood velocity after vehicle treatment was significantly decreased by 30% at 4 wk, compared with sham-operated mice (Figure 6C), and after COMP-Ang1 treatment, peak renal artery blood velocity was significantly increased 1.7-fold at 4 wk, compared with vehicle-treated mice (Figure 6C). These results indicate that COMP-Ang1 increases renal cortical blood flow and peak renal artery blood velocity in UUO mice.

F4/80-Positive Cell Infiltration Is Decreased after COMP-Ang1 Treatment

The number of monocytes/macrophages, as identified by the immunohistochemistry of F4/80 antigen per unit area in vehicle-treated kidneys, increased seven- to 10-fold for 1 and 2 wk after UUO, respectively, compared with sham-operated kidneys (Figure 7, A and B). COMP-Ang1 significantly decreased the number of F4/80-positive cells by 31 and 45% at 1 and 2 wk, respectively, compared with vehicle-treated UUO kidneys (Figure 7, A and B). Pretreatment with sTie2-Fc reversed the effect of COMP-Ang1 in the infiltrating F4/80-positive cells (Figure 7C). Thus, COMP-Ang1 treatment decreased the monocyte/macrophage infiltration through Tie2 in UUO kidneys.

Changes in Renal TGF-β1, Smad 2/3, and Smad 7 after COMP-Ang1 Treatment

TGF-β1 has a pivotal role in renal fibrosis in animal studies as well as human clinical specimens, and the number of macrophages in the interstitium and renal cortical TGF-β1 mRNA levels are highly correlated (31,32). Quantification by a specific ELISA showed tissue levels of active TGF-β1 to be significantly increased in vehicle-treated UUO kidneys (Figure 7D). After COMP-Ang1 treatment, renal TGF-β1 was significantly decreased by 48, 31, and 35% at 5, 7, and 14 d after UUO, respectively, compared with vehicle treatment (Figure 7D).

Downregulation of Smad 3 is associated with attenuated renal fibrosis in the UUO model, and Smad 7 negatively regulates renal fibrosis by inhibiting Smad 2/3 activation in vivo (33–35). Immunobots showed that compared with sham-operated mice, renal phospho-Smad 2 and 3 increased 4.1- and 2.9-fold, respectively, after 14 d in vehicle-treated UUO kidneys (Figure 7E). After COMP-Ang1 treatment, phospho-Smad 2 and 3 decreased approximately 29 and 41%, respectively, in UUO kidneys after 14 d compared with treatment with vehicle (Figure 7E). After 14 d, Smad 7 was decreased approximately 42% in vehicle-treated UUO kidneys compared with sham-
109 pfu Ade-sTie2-Fc 24 h before treatment with sTie2-Fc at 1 wk after surgery. Mice were pretreated with COMP-Ang1 at 1 wk after surgery. Bar = 50 μm. (B) The number of infiltrating F4/80-positive cells in kidneys treated with vehicle or COMP-Ang1 at 1 and 2 wk after UUO (n = 6 for each experimental group). Data are expressed as means ± SD. Gray bars indicate vehicle treatment; red bars indicate COMP-Ang1 treatment. (C) The number of infiltrating F4/80-positive cells in UUO kidneys treated with COMP-Ang1 with or without sTie2-Fc at 1 wk after surgery. Mice were pretreated with 1 × 10^9 pfu Ade-sTie2-Fc 24 h before treatment with 1 × 10^9 pfu Ade-COMP-Ang1 (n = 5 for each experimental group). (D) The tissue level of activated TGF-β1 was quantified by ELISA in sham-operated or UUO kidneys at 5, 7, and 14 d after surgery. Results were similar from four independent experiments. (E and F) Immunoblot analyses of phosphorylated Smad 2/3 (E) and Smad 7 (F) in UUO kidneys treated with COMP-Ang1 or vehicle. Blots were probed with phospho-Smad 2/3 or Smad 7 antibodies. The membrane was stripped and reprobed with anti-actin antibody to verify equal protein loading. Results were similar from four independent experiments. Densitometric analyses are presented as the relative ratio of phospho-Smad 2/3 or Smad 7 to actin. The relative ratio measured in sham-operated kidneys is arbitrarily presented as 1. Data are expressed as mean ± SD. *P < 0.05 versus sham-operated mice treated with vehicle; †P < 0.05 versus UUO kidneys treated with vehicle on the same day; ‡P < 0.05 versus sham-operated mice treated with COMP-Ang1; §P < 0.05 versus UUO kidneys treated with vehicle on the same day; ††P < 0.05 versus UUO kidneys treated with COMP-Ang1 on the same day.

Figure 7. COMP-Ang1 decreases the number of infiltrating F4/80-positive cells and regulates TGF-β1 and Smad in UUO kidneys. (A) Representative light micrographs of F4/80 staining within kidney sections from vehicle- or COMP-Ang1–treated mice at 1 wk after surgery. (B) The number of infiltrating F4/80-positive cells in the kidneys treated with vehicle or COMP-Ang1 at 1 and 2 wk after UUO (n = 6 for each experimental group). Data are expressed as means ± SD. Gray bars indicate vehicle treatment; red bars indicate COMP-Ang1 treatment. (C) The number of infiltrating F4/80-positive cells in UUO kidneys treated with COMP-Ang1 with or without sTie2-Fc at 1 wk after surgery. Mice were pretreated with 1 × 10^9 pfu Ade-sTie2-Fc 24 h before treatment with 1 × 10^9 pfu Ade-COMP-Ang1 (n = 5 for each experimental group). (D) The tissue level of activated TGF-β1 was quantified by ELISA in sham-operated or UUO kidneys at 5, 7, and 14 d after surgery. Results were similar from four independent experiments. (E and F) Immunoblot analyses of phosphorylated Smad 2/3 (E) and Smad 7 (F) in UUO kidneys treated with COMP-Ang1 or vehicle. Blots were probed with phospho-Smad 2/3 or Smad 7 antibodies. The membrane was stripped and reprobed with anti-actin antibody to verify equal protein loading. Results were similar from four independent experiments. Densitometric analyses are presented as the relative ratio of phospho-Smad 2/3 or Smad 7 to actin. The relative ratio measured in sham-operated kidneys is arbitrarily presented as 1. Data are expressed as mean ± SD. *P < 0.05 versus sham-operated mice treated with vehicle; †P < 0.05 versus UUO kidneys treated with vehicle on the same day; ‡P < 0.05 versus sham-operated mice treated with COMP-Ang1; §P < 0.05 versus UUO kidneys treated with vehicle on the same day; ††P < 0.05 versus UUO kidneys treated with COMP-Ang1 on the same day.

Discussion
We demonstrated here that less tubular injury and tubulointerstitial fibrosis occurred in UUO mice that received COMP-Ang1 than in those that received vehicle. The accumulation of α-SMA–positive cells and interstitial collagen type I was significantly suppressed by COMP-Ang1 treatment. COMP-Ang1 preserved renal PECAM-1– and Tie2-positive EC and increased Akt phosphorylation. Renal surface microvasculature increased in vivo and increased renal blood flow increased in laser Doppler ultrasonography after COMP-Ang1 treatment compared with vehicle treatment. COMP-Ang1 decreased monocyte/macrophage infiltration, tissue levels of TGF-β1, and Smad 2/3 phosphorylation and increased Smad 7 in UUO kidneys. All of these findings demonstrate that COMP-Ang1 treatment can retard the progression rate of renal fibrosis in ureteral obstruction.

The response of PECAM-1–positive EC to COMP-Ang1 differed between 2 and 4 wk after surgery. After 2 wk, PECAM-1–positive peritubular EC in vehicle-treated UUO kidneys were slightly increased compared with sham-operated kidneys. COMP-Ang1 administration increased expression of PECAM-1 in cortical peritubular capillaries in UUO kidneys 2 wk after surgery compared with vehicle treatment. Because renal peritubular capillary density was increased in UUO kidneys that were treated with COMP-Ang1 compared with sham-operated or vehicle-treated UUO kidneys, we propose that COMP-Ang1 induces angiogenesis 2 wk after UUO. After 4 wk, PECAM-1–positive peritubular EC decreased in vehicle-treated UUO kidneys compared with sham-operated kidneys. The expression of PECAM-1 in peritubular capillary EC was increased in COMP-Ang1–treated kidneys compared with vehicle-treated UUO kidneys at 4 wk after UUO. Although renal peritubular capillary density increased in UUO kidneys after COMP-Ang1 treatment compared with vehicle-treated UUO kidney, it was not increased compared with sham-operated mice. Thus, COMP-Ang1 has a protective effect on renal capillary EC 4 wk after surgery.

Infiltrating inflammatory cells, such as monocytes/macrophages, release cytokines that can induce collagen synthesis by fibroblasts and cause further fibrosis (36,37). We previously demonstrated that Ang1 has an anti-inflammatory effect by reducing vascular endothelial growth factor–induced endothelial adhesiveness (15). Here, we demonstrate that COMP-Ang1 treatment decreases the UUO-induced increase in F4/80-positive monocyte/macrophage infiltration in the kidney. Decreased interstitial macrophage infiltration was correlated with reduced tubulointerstitial fibrosis in the UUO model (36). These results suggest that COMP-Ang1 can be an anti-inflammatory agent in the regulation of macrophage infiltration in UUO.

Endothelial stabilization by COMP-Ang1 is achieved in part by secondary activation of pericytes. Therefore, we examined interaction between EC and pericytes in the UUO model. In an examination of double-immunostained EC and pericytes 2 wk after treatment, COMP-Ang1 did not increase pericyte recruitment in the UUO model (Supplementary Figure 1). We also
evaluated changes in levels of endogenous Ang1 and Ang2 in UUO or contralateral kidneys at 5, 7, and 14 d after surgery. Ang1 protein levels decreased 7 and 14 d after surgery, and Ang2 protein levels decreased 5, 7, and 14 d after surgery (Supplementary Figure 2). We observed no significant change in body weight, blood urea nitrogen, or urine protein/creatinine ratio between vehicle and COMP-Ang1–treatment groups 2 and 4 wk after surgery (Supplementary Table 1).

Although it is impossible to compare precisely the protective effect of multiple angiogenic factors in the UUO model, the COMP-Ang1 effect on tubular injury is comparable to that of bone morphogenetic protein-7 (10) and IGF-1 (11) and less potent than that of hepatocyte growth factor (9). We also found that the protective effect of COMP-Ang1 on tubulointerstitial morphology as indicated by periodic acid-Schiff stain is not significantly different in mice that were treated with COMP-Ang1 3 d before surgery and mice that were treated after surgery (data not shown). We suggest that COMP-Ang1 has a therapeutic and prophylactic effect as based on the UUO model.

**Conclusion**

COMP-Ang1 largely protects obstructed kidneys by significantly improving morphology, interstitial fibrosis, and type I collagen deposition. These results suggest that COMP-Ang1 may be a novel, endothelium-specific therapeutic modality in fibrotic renal disease.

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**References**


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See the related editorial, “Protecting the Microvasculature: A Tight Connection to Ameliorating Chronic Kidney Disease?” on pages 2353–2355.