Effect of combination therapy with enalapril and the TGF-β antagonist 1D11 in unilateral ureteral obstruction

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El Chaar M, Chen J, Seshan SV, Jha S, Richardson I, Ledbetter SR, Vaughan ED Jr, Poppas DP, Felsen D. Effect of combination therapy with enalapril and the TGF-β antagonist 1D11 in unilateral ureteral obstruction. Am J Physiol Renal Physiol 292: F1291–F1301, 2007. First published December 12, 2006; doi:10.1152/ajprenal.00327.2005.—In unilateral ureteral obstruction (UUO), the kidney is characterized by increased fibrosis and apoptosis. Both transforming growth factor-β (TGF-β) and ANG II have been implicated, and ANG II may mediate its effects through TGF-β. Previous studies demonstrated amelioration of renal damage when either TGF-β or ANG II has been individually targeted. In this study, we sought to determine whether combining 1D11 (monoclonal antibody to TGF-β) and an ACE inhibitor, enalapril, would be more effective in UUO than either individual treatment, as has been shown in diabetic and glomerulonephritic models. Rats underwent UUO and were given either control monoclonal antibody, 1D11 or enalapril, or 1D11/enalapril combination, for 14 days. Kidneys were harvested and examined for fibrosis (trichrome; collagen (real-time PCR, Sircol assay) and fibroblast-specific protein expression (immunohistochemistry), apoptosis (TUNEL), macrophage infiltration (immunohistochemistry), and TGF-β expression (real-time PCR and tubular localization with immunohistochemistry)). UUO was found to induce fibrosis, apoptosis, macrophage infiltration, and TGF-β expression in the obstructed kidney. Administration of either 1D11 or enalapril individually significantly decreased all these changes; when 1D11 and enalapril were combined, there was little additive effect, and the combination did not provide full protection against damage. The results demonstrate that, for the most part, combination therapy is not additive in UUO. This could be due to the continued presence of a physical obstruction or to biochemical differences between UUO and other renal disease models. Furthermore, it suggests that other targets may be amenable to pharmacological manipulation in UUO.

Fibrosis; apoptosis; transforming growth factor-β; converting enzyme inhibitor

IN UNILATERAL URETERAL OBSTRUCTION (UUO), the kidney is characterized by increased fibrosis and apoptosis, along with decreases in renal function (6, 12). Transforming growth factor-β, a proapoptotic and profibrotic cytokine, has been implicated in these changes. Both an antibody to TGF-β (1D11) and antisense nucleotides directed toward TGF-β have been shown to protect the obstructed kidney and ameliorate renal damage (7, 20). When individual isoforms of TGF-β have been analyzed for effects on fibrosis, it has been found for the most part that TGF-β1 and TGF-β2 are profibrotic, whereas TGF-β3 may reduce scarring (21, 37).

ANG II has also been considered an important factor in UUO. Studies with either angiotensin-converting enzyme (ACE) inhibitors or an ANG II receptor antagonist have demonstrated that fibrosis can be ameliorated by these drugs (13). Furthermore, in studies on mice in which one to four copies of the angiotensinogen were expressed, there was a direct correlation between copy number and fibrosis (4). ANG II has been implicated in the progression of the kidney to end-stage renal disease in diabetes and other chronic renal diseases (16, 25).

In addition to accumulation of collagen, UUO is also characterized by an increase in renal fibroblasts and myofibroblasts. Fibroblasts may arise through epithelial mesenchymal transition (EMT), an important biological process in which epithelial cells lose their epithelial phenotype and instead express characteristics associated with mesenchymal cells (31, 38). EMT includes a loss of shape and polarity, and loss of expression of proteins associated with tight junctions, desmosomes, and cytokeratin intermediate filaments (31, 38), along with increased expression of fibroblast-specific protein (FSP-1), α-smooth muscle actin (α-SMA), vimentin, collagen, fibronectin, and others reflective of their new phenotype (10). Further evidence of FSP-1 as a specific fibroblast marker was demonstrated when Okada et al. (22) showed in vitro that renal proximal tubular cells incubated with TGF-β showed morphological and biochemical changes consistent with EMT, along with FSP-1 expression. Thus TGF-β may be important for induction of fibrosis and transition of epithelial cells to fibroblasts.

Neither 1D11 by itself nor treatments which reduce ANG II has been shown to totally protect the obstructed kidney from damage. In a model of diabetic nephropathy, it was shown that the combination of 1D11 and lisinopril (ACE inhibitor) fully arrested both proteinuria and renal injury, whereas neither treatment by itself was totally effective (2). Likewise, in a model of glomerulonephritis, combination of enalapril and 1D11 had an enhanced antifibrotic effect, compared with the two drugs given separately (36). Therefore, we sought to determine whether a combination of 1D11 and the ACE inhibitor, enalapril, would be more effective in UUO than either treatment by itself. We used a 14-day model of UUO and examined renal fibrosis, apoptosis, macrophage infiltration, and fibroblast expression, along with examination of tissue expression of all three isoforms of TGF-β.
In vivo UUO. Sprague-Dawley rats (n = 8 per group) underwent unilateral ureteral ligation with 4–0 silk suture through a midline abdominal incision under sterile conditions as routinely carried out in our lab (8). Enalapril was administered in drinking water at 200 mg/l. Drinking water was supplemented with enalapril 1 day before UUO. ID11 (0.5 mg/kg) was administered by intraperitoneal injection 1 day before UUO and every day thereafter. An antiserotokxin antibody, 13C4, was administered (0.5 mg/kg) as a control. Kidneys were harvested at day 14. Animal treatment adhered to approved institutional guidelines.

Renal histology. Masson trichrome-stained sections of paraffin-embedded specimens were examined and analyzed by a board-certified pathologist (S. V. S., renal pathology specialist) who examines hundreds of clinical cases per year. The extent and intensity of staining in the renal cortex and medulla were semiquantitatively scored on a scale of 0–3+.

Tissue collagen. Total soluble collagen concentration was measured with the Sircol collagen assay kit (Accurate Chemical and Scientific) according to the manufacturer’s protocol. In brief, dry tissues were dissolved in 0.5 M acetic acid and heated for 120 min at 60°C. Tissue suspensions were centrifuged, supernatants were collected, and total collagen was measured at 540 nm.

Total RNA extraction and cDNA synthesis. Kidneys stored in RNAlater (Ambion) were homogenized in 0.5 ml of TRIzol reagent (GIBCO) and processed using the RNAeasy Mini Kit (Qiagen), per manufacturer’s directions. RNA samples were dissolved in 50 μl of nuclease free water and then digested with DNase I (Ambion) to avoid nonspecific fluorescence emission derived from the recognition of contaminating genomic DNA by the probe. cDNA was generated from 3–5 μg of RNA by using murine MLV reverse transcriptase first-strand cDNA synthesis kit (Pharmacia).

Real-time PCR for TGF-β isoforms and collagen. PCR reactions were performed and monitored using an ABI Prism 7700 Sequence Detection system (Perkin-Elmer Applied Biosystem). The PCR master mix was based on AmpliTaq Gold DNA polymerase (PE-A-P); cDNA samples were analyzed in triplicate. Primers and probes were used at concentration of 100 and 125 nM per reaction, respectively.

After an initial denaturation step of 95°C for 10 min, the cDNA products were amplified with 40 PCR cycles (denaturation: 95°C for 15 s; extension: 60°C for 1 min). Data analysis was carried out using the sequence detector V program (PE-A-P). For each sample, the Ct value was determined as the cycle number at which the fluorescence intensity reached 0.05; this value was chosen after confirming that in this range all curves were in the exponential phase of amplification.

Immunofluorescence for TGF-β isoforms. The expression of TGF-β1, TGF-β2, and TGF-β3 was assessed by indirect immunofluorescence staining of formalin-fixed 4-μm paraffin sections using specific polyclonal antibodies against TGF-β1, TGF-β2, and TGF-β3 (Santa Cruz Biotechnology, Santa Cruz, CA). Sections were deparaffinized and treated with antigen unmasking solution (Vector) containing 1% SDS and 0.1 M citrate buffer at pH 6.0 for 5 min, then cooled down at room temperature for 15 min. Trypsin at the concentration of 0.05% was used to digest the nonspecific protein deposition and formaldehyde bonds. Nonspecific background was blocked with 1% bovine albumin serum in PBS for 15 min at room temperature. Endogenous fluorescence was blocked with TNB buffer, and sections were incubated overnight at 4°C with anti-TGF-β1, anti-TGF-β2, or anti-TGF-β3 antibodies (1:200 dilution in TNB buffer). Bound antibody was detected using horseradish peroxidase-anti-rabbit antibody and fluorescein (FITC)-labeled tyramide in the dilution of 1:50 with amplification solution. The sections were viewed and photographed using an Olympus BX40 binocular microscope and an Olympus DP 70 digital camera. There was no immunostaining in control sections in which primary antibody was omitted or when primary antibody was substituted by preimmune rabbit serum. In addition, preabsorbing the antisem with TGF-β1, TGF-β2, or TGF-β3-specific peptides abolished binding to tissue sections.

Immunohistochemical analysis. Immunohistochemical analyses for macrophages were performed and monitored using a monoclonal antibody to ED-1 (Serotech) as previously described (8). Macrophages were counted in 10 high-power fields (×400) by two different independent investigators in a blinded fashion. The results were averaged and are presented in Table 1, along with results for TUNEL and FSP. The TUNEL assay was performed as previously described (8), and TUNEL-positive apoptotic renal tubules were quantitated as above.

We also analyzed tissue for the presence of fibroblasts using immunohistochemistry, as previously described (8). The antibody utilized was DAKO S100-A4 (1:100 dilution). The S100-A4 antigen is also known as FSP-1 (1, 32). Antigen was retrieved by incubating cells with proteinase K for 20 min in an oven. The remaining immunoperoxidase protocol was carried out as routinely done in our laboratory. Staining for S100-A4 was found in spindle-shaped interstitial cells and also in cells which were round and were identified as inflammatory cells by the pathologist. Only spindle-shaped cells were included in the counts and were counted as above. Samples incubated without primary antibody exhibited no staining.

Renal tubular segments were identified using immunohistochemistry in paraffin sections. Proximal tubules were visualized with an antibody to Lotus Tetranoglobus lectin (Vector; 1:100 dilution); distal tubules, including thick ascending limb, were visualized with antibody to arachis hypogea (peanut lectin; Dako Labs; 1:100) and distal tubules (particularly thin limb of Henle and collecting ducts) were visualized with pan-cytokeratin antibody (Santa Cruz Biotechnology; 1:100).

Statistics. Samples were analyzed by one-way ANOVA, with post hoc analysis using a Dunnett’s test, and differences were considered significant at P < 0.05.

RESULTS

Animals were subjected to UUO for 14 days, and kidneys were harvested at that time. The animals were treated with either a control monoclonal antibody, 1D11, enalapril, or 1D11 + enalapril, as detailed in MATERIALS AND METHODS. We examined several parameters of renal damage. All obstructed kidneys (OK) were compared with contralateral unobstructed kidney (CK). Although the CK is not normal, it is used for comparison; for the most part, the CK exhibits very little damage as noted in the data presented below, where there are very few apoptotic cells, macrophage infiltration, or fibrosis in the CK.

Apoptosis. In the control OK, 2 wk of UUO resulted in a significant increase in apoptotic tubular cells compared with the CK. In the CK, there were 0.2 ± 0.0 apoptotic cells/HPF.
compared with 7.5 ± 0.8 apoptotic cells/HPF in the OK. Both 1D11 and enalapril treatment alone significantly decreased apoptosis in the OK (Fig. 1 and Table 1). Combined treatment with 1D11 and enalapril did not further decrease apoptosis in the OK.

Macrophage infiltration. Similarly, there was a significant increase in macrophage infiltration into the OK compared with the CK after 2 wk of UUO (28.2 ± 3.2 vs. 2.2 ± 0.4 cells/HPF, respectively). Both 1D11 and enalapril significantly decreased macrophage infiltration into the OK (Fig. 2 and Table 1). In this case, the combination of 1D11 and enalapril caused a further significant decrease of macrophages in the OK. However, there were still significantly more macrophages in the OK treated with the drug combination, compared with the CK.

Fibroblast expression. We examined fibroblast infiltration into the kidney using the S100A antibody. There was a small number of interstitial fibroblasts present in control kidneys (5.3 ± 0.2 FSP⁺ cells/HPF). Fourteen-day UUO increased this number to 34.8 ± 0.8 cells/HPF. 1D11 decreased the amount of fibroblast infiltration by almost 50%, whereas enalapril decreased the fibroblast expression slightly less (Fig. 3 and Table 1). The combination of 1D11 and enalapril caused a further decrease, but there were still significantly more fibroblasts in the OK, compared with the CK.
Renal collagen. Renal collagen mRNA was measured with real-time PCR. Using these measurements, there was a significant increase in collagen mRNA in the OK compared with the CK (Fig. 4A). 1D11, enalapril, or the combination each caused a significant decrease in renal collagen mRNA, but none of the groups was significantly different from each other.

Total renal collagen was measured using the Sircol assay. Baseline renal collagen was 29.5 ± 1.6 μg/mg tissue (Fig. 4B). Collagen was significantly increased by UUO to 43.2 ± 3.2 (μg/mg tissue), as has previously been shown in obstruction. Both enalapril and 1D11 significantly decreased renal collagen, both by almost 20%. The combination of 1D11 and enalapril also significantly decreased renal collagen but was not more effective than either drug alone.

Analysis of trichrome staining for interstitial fibrosis. CK showed very little, if any, inflammation in tubules, glomeruli, or interstitial fibrosis (Fig. 4C). The OK of the control group showed moderate (1 ± 2) positive medullary staining, along with focal peripelvic areas of 1 + staining (Fig. 4C). The cortex showed less fibrosis than the medulla. Enalapril-treated rat kidneys demonstrated decreased trichrome staining, compared with control, being trace to focal +1. 1D11 in this experiment showed modest effects on medullary fibrosis, and the combination of both drugs was similar to enalapril alone.

Renal TGF-β mRNA expression. We used real-time PCR to examine expression of the three isoforms of TGF-β. The relative expression of TGF-β1 mRNA is shown in Fig. 5A. As seen, there was a significant increase in TGF-β1 mRNA in the OK, compared with the CK. Both enalapril and 1D11 significantly decreased TGF-β1 mRNA in the OK, compared with obstruction alone. The combination of 1D11 and enalapril further significantly decreased TGF-β1 mRNA compared with enalapril, but not to 1D11 treatment. All treated groups were significantly different from the CK.

There was also a significant increase in TGF-β2 mRNA in the OK compared with the CK (Fig. 5B). In the case of TGF-β2 mRNA, all treatments significantly decreased TGF-β2 mRNA to values that were not significantly different from the CK or each other.

There was a significant increase in TGF-β3 mRNA in the OK compared with the contralateral (Fig. 5C). There were no significant differences between the OK and any of the treated OK. With TGF-β3, there was considerable scatter of the data, as well as overlap between groups.

Immunohistochemical localization of TGF-β isoforms. We examined the expression of all three isoforms of TGF-β in renal tissue. In Fig. 6A (TGF-β1), B (TGF-β2), and C (TGF-β3), the typical immunofluorescent staining pattern is shown of each isoform of TGF-β in contralateral unobstructed and obstructed kidneys, in control and treated groups. To localize the TGF-β staining to individual tubular segments, we used specific lectin/antibody staining as described in MATERIALS AND METHODS. These are shown in Fig. 6, D and E. In Fig. 6D, a typical staining is shown of renal tubular segments in contralateral and control obstructed kidneys. Immunoperoxidase staining using lectins [lotus tetragonolobus (LN) and peanut lectin (PL)] and monoclonal antibody to pan cytokeratin [pan-Cyt K] demonstrated positive tubular cell staining for proximal tubules with LT, distal tubules with PL, and thin loop of Henle with pan-Cyt K in the contralateral kidney. In the control UUO kidney, almost all the affected (dilated) segments were identi-
**Expression of Collagen III in UUO model**

- **A**
  - Relative mRNA levels for different groups:
    - Control
    - Eileapril
    - 1D11
    - Eileapril + 1D11
  - Unobstructed and Obstructed conditions

- **B**
  - Renal Collagen [µg/mg tissue]
  - Groups:
    - Control
    - 1D11
    - Eileapril
    - 1D11/Eileapril
  - Bar charts showing differences among groups

- **C**
  - Histological images:
    - CLK
    - Control OK
    - 1D11-treated
    - Enalapril-treated
    - 1D11 + Enalapril treated
Contralateral and obstructed kidney.

Fig. 6. Effect of 1D11, enalapril, or their combination on tissue TGF-β expression in UUO. Rats were treated as in MATERIALS AND METHODS and kidneys were harvested at day 14. TGF-β mRNA expression was assessed as described in MATERIALS AND METHODS. 

DISCUSSION

1D11 is a monoclonal antibody which is active against all three isoforms of TGF-β. It has been used as a single agent in
A

Contra.

UO

Control ACE-i 1D11 1D11 + ACE-i

B

Contra.

UO

Control ACE-i 1D11 1D11 + ACE-i

C

Contra.

UO

Control ACE-i 1D11 1D11 + ACE-i
1D11 (TGF-β ANTAGONIST) AND ACE INHIBITION IN UUO

D

Control

UUO

Peanut lectin
Pan-Cyt K
Lotus

E

TGF β1
TGF β2
TGF β3

UUO
Peanut lectin
Pan-Cyt K
Lotus
several models of renal disease including UUO, diabetes, glomerulonephritis, and cyclosporine nephropathy (2, 18, 20, 36). Similarly, either ACE inhibitors or ANG II antagonists have been used both clinically and experimentally in both renal and cardiac disease models (13, 16, 17, 25, 30). It was shown that the combination of both 1D11 and an ACE inhibitor provided superior protection in either experimental diabetes or glomerulonephritis, than either drug alone (2, 36). In the present study, we sought to determine whether a combination of 1D11 and the ACE inhibitor, enalapril, would provide more protection against renal damage in UUO than each drug used separately. We found that each drug individually provided some protection against several parameters of renal damage, including apoptosis, macrophage infiltration, and fibrosis and fibroblast expression. The combination of the two drugs did not, for the most part, enhance their activities nor did it provide complete protection against renal damage in UUO. This suggests that there may be underlying differences in the mechanisms which result in the renal damage in UUO compared with other renal diseases.

This study confirmed our previous findings in which 1D11 was used at a higher dose in UUO (20). We demonstrated its effectiveness as an inhibitor of apoptosis; furthermore, we demonstrated effects on fibrosis by measurements of tissue collagen and collagen mRNA expression. We also extended our study to include effects of 1D11 on macrophage infiltration, fibroblast expression, and TGF-β expression.

1D11 had significant effects on both mRNA and protein expression of all three TGF-β isoforms. 1D11 significantly decreased TGF-β1 mRNA expression in the OK compared with control obstructed kidney. It also significantly decreased TGF-β2 expression but had no effect on TGF-β3 expression in the OK. These results confirm the known effects of TGF-β as an activator of its own expression. There are two sites on the promoter of the TGF-β1 gene which are responsive to autoregulation by TGF-β (11). Effects of 1D11 on TGF-β2 mRNA expression suggest that it too is subject to regulation by TGF-β. When TGF-β expression by immunofluorescence was examined, the effects of 1D11 on TGF-β3 mRNA expression were confirmed; however, effects on TGF-β1 and TGF-β2 protein expression were more pronounced than on mRNA. In 1D11-treated rats, most of the TGF-β1 and TGF-β2 staining was absent, whereas TGF-β3 staining was relatively unchanged in intensity. This demonstrates the importance of assessing tissue protein levels of TGF-β.

TGF-β has long been known to have anti-inflammatory properties (15). Mice having a targeted deletion of the TGF-β gene die with multiorgan inflammation, along with other effects including expression of adhesion molecules effects on leukocyte proliferation and differentiation (15). However, the response to TGF-β may depend on a variety of factors. In studies on cardiac fibrosis, Koyanagi et al. (14) using a monoclonal antibody against TGF-β2 blocked cardiac inflammation, induced by Nω-nitro-L-arginine methyl ester treatment, suggesting a proinflammatory role of TGF-β or its downstream mediators. In the present studies, 1D11 significantly decreased macrophage infiltration into the kidney in UUO, suggesting that TGF-β contributes to the inflammatory effect in UUO.

We also demonstrated effects of 1D11 on fibroblasts in the OK. Although TGF-β has been shown to be a strong inducer of EMT of renal epithelial cells in vitro (22), there had been little direct evidence in vivo that it is responsible for this transition. Fibroblasts in the OK may be native or may arise from renal tubule cells following EMT (9). The transition of epithelial cell to fibroblast has been confirmed by expression of either α-smooth muscle actin or FSP. Several studies have examined EMT in UUO. Yang and Liu (35) showed a marked decrease in e-cadherin in UUO and a marked increase in α-SMA. There was also an increase in TGF-β receptors in renal tubules, as was previously demonstrated by our laboratory (33). Iwano et al. (9) used a combination of bone marrow chimeras and transgenic reporter mice and studied the expression of FSP+ cells in UUO. A large proportion of FSP+ cells arose from local EMT of proximal tubular cells. In the present study, we used an antibody to antigen S100A4 to identify fibroblasts. It is known that SFP-1 is expressed in interstitial fibroblasts and that FSP-1 is identical to S100A4 (1). S-100A4 is a calcium binding protein which has been implicated in invasion and metastasis of several tumor lines. We identified the spindle-shaped interstitial cells immunolocalized by S100-A4 as fibroblasts. As noted, 1D11 treatment decreased expression of S-100A4-positive cells in the OK. Therefore, the results presented here provide supportive evidence for the TGF-β dependency of EMT.

Enalapril had significant effects on the same parameters as described above. To about the same degree as 1D11, enalapril was able to block apoptosis, collagen synthesis, macrophage infiltration, and FSP expression. ANG II has known effects on collagen synthesis, apoptosis, and macrophage infiltration. Systemic ANG II infusion into rats results in inflammatory cell infiltration into the kidney (27). ANG II also regulates matrix production in vitro in cultured renal cells and has been shown to directly induce apoptosis in HUVEC (3, 34).

Enalapril had effects similar to 1D11 on TGF-β mRNA expression. However, it had different effects from 1D11 on tissue TGF-β protein expression. It almost completely inhibited TGF-β1 expression but partial to persistent TGF-β2 and TGF-β3 expression was present.

Although ANG II has direct effects in vitro which are profibrotic and proinflammatory, its effects may also be mediated by TGF-β. In vitro, ANG II induces TGF-β release in renal tubular cells (19). ANG II-induced stimulation of collagen IV synthesis in tubular cells (34) and ANG II-induced ECM production in glomerular cells are both mediated by TGF-β (5). Similar effects have been noted with fibroblasts and vascular smooth muscle cells (26, 28). In the present experiments, we combined a TGF-β antibody 1D11 with enalapril, an ACE inhibitor. In the diabetes model in which this strategy was employed, there was a clear additive effect of 1D11 and the ACE inhibitor lisinopril (2). When urinary protein excretion or collagen III staining was examined, the combination of 1D11 and lisinopril was significantly more effective than either drug used alone. Similarly glomerular morphology was protected by the drug combination. In the glomerulonephritis model, an additive effect of the two drugs was noted, especially on fibrosis (36). In that study, enalapril was used at the same dose used in our study, 1D11 was used at either 0.5 or 5 mg/kg; we used 1D11 at 0.5 mg/kg.

In this study, we localized TGF-β expression to specific tubular segments using lectins or monoclonal antibody. UUO...
was associated with an increase in intensity of staining of all three TGF-β isoforms in various segments of the nephron. In UUO, TGF-β1 was localized mainly in diluted distal tubules, as was TGF-β2. TGF-β3 was localized to distal tubules but also to the brush border of proximal tubules in UUO. The combination of 1D11 and enalapril eliminated TGF-β1 staining. For TGF-β2, a small proportion of both small/dilated distal segments of tubules (~10%) showed persistent staining, and for TGF-β3 segmental trace-2+ tubular cell staining was noted in all dilated distal tubules. It has been suggested that TGF-β3 can prevent scarring (29), and thus increased TGF-β3 could contribute to an antifibrotic effect of both 1D11 and enalapril. However, Yu and colleagues (37) studied the effect of all three isoforms of TGF-β in vitro in three different renal cell types and concluded that all three isoforms are fibrogenic in kidney. Therefore, it is unclear whether elevated TGF-β3 in the kidney would have antifibrotic effects.

In the present study, we saw little additive effects of the two drugs. Since UUO begins as a mechanical insult to the kidney, compared with the metabolic or immunological changes in diabetes or in glomerulonephritis, and the outlet obstruction remains in place during treatment, this could contribute to the differences between the response of UUO and that of other renal diseases. In addition, UUO is characterized predominantly by interstitial changes, whereas in diabetes and glomerulonephritis, there is significant glomerular involvement; this could also contribute to the differences noted. The additive effect on macrophage infiltration was found in the diabetes model (2). In the glomerulonephritis model, there was no additive effect at the 0.5-mg/kg 1D11 dose. The higher dose of 1D11 was less effective in inhibiting macrophage infiltration, and this effect was reversed by enalapril. It has previously been shown that inhibition of ANG II decreases TGF-β in experimental models of renal injury. For example, it was shown that losartan administration was accompanied by suppression of the increased TGF-β in UUO (24). Peters et al. (23) demonstrated similar findings in the anti-Thy 1 model of glomerulonephritis. In diabetic patients treated with the ACE inhibitor captopril, serum TGF-β was decreased, and renoprotection was seen (30). The present results suggest that in this experimental model both 1D11 and enalapril are targeting the same pool of TGF-β.

In summary, this study examined the effects of either 1D11, enalapril, or a combination of the two drugs on renal damage, fibrosis, and TGF-β expression. Although it had been shown in other models that the combination of 1D11 and enalapril was more effective than either drug alone, our results suggest that in UUO this is not the case; furthermore, much of the damage (as measured by fibrosis, apoptosis, and macrophage infiltration) is still present even with drug treatment. Renal damage persists despite a significant decrease in both TGF-β1 and TGF-β2, as seen with immunohistochemical staining. These results suggest that other targets should be identified and may be amenable to pharmacological manipulation in UUO.

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