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A novel inhibitor of tumor necrosis factor-α converting enzyme ameliorates polycystic kidney disease

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Background. Transforming growth factor-α (TGF-α) expression is abnormal in polycystic kidney disease. We previously demonstrated that blockade of the epidermal growth factor receptor (EGFR), the receptor for TGF-α, significantly slowed disease progression in the *bpk* murine model of autosomal-recessive kidney disease (ARPKD). In the present study, kidney TGF-α expression in this model is characterized, and the therapeutic potential of inhibiting TGF-α in ARPKD is examined using a novel inhibitor of tumor necrosis factor-α converting enzyme (TACE), the metalloproteinase that cleaves membrane-bound TGF-α to release the secreted ligand.

Methods. Immunohistochemistry (IH) and Western analysis were performed on kidneys from cystic *bpk* mice and noncystic littermates at postnatal days 7, 14, and 21. *Bpk* mice and normal controls were treated with WTACE2, a competitive inhibitor of TACE, from day 7 until day 21, and the effects on kidney histology and renal function were assessed.

Results. Increased TGF-α expression by IH was demonstrated in the proximal tubules (PT) at postnatal day 7 and collecting tubules (CT) by day 21. A parallel increase in kidney TGF-α expression was demonstrated by Western analysis. Treatment of cystic *bpk* mice with WTACE2 resulted in a 43% reduction in kidney weight to body weight ratio (11.2 vs. 19.7%), improved cystic index (3.2 vs. 4.8), reduced cystic CT to PT ratio (1.2 vs. 8), and a greater than 30% reduction in BUN and serum creatinine.

Conclusions. These findings support the pathophysiological role of the TGF-α/EGFR axis in murine ARPKD and demonstrate that inhibition of TGF-α secretion has therapeutic potential in PKD.

Autosomal-recessive polycystic kidney disease (ARPKD) is an inherited disorder that usually presents in the newborn period with massive kidney enlargement (due to rapidly expanding cysts) and hepatic fibrosis. ARPKD occurs in approximately 1:10,000 to 1:40,000 births and produces significant morbidity and mortality [1]. Data from experimental models of both recessive and dominant forms of PKD have identified three key pathophysiologic processes in cyst formation and enlargement: increased cell proliferation, increased fluid secretion, and altered matrix biology [reviewed in 2]. A growing body of evidence has established the central role of the epidermal growth factor receptor (EGFR) in the pathogenesis of cell proliferation in PKD. The EGFR in cystic epithelia is overexpressed and mislocalized to the apical surface in both ARPKD and ADPKD [3–5]. Apically expressed EGFR is capable of binding ligand and transmitting mitogenic signals [6].

Published reports also have suggested that transforming growth factor-α (TGF-α), a ligand of the EGFR, is abnormally expressed in PKD. TGF-α expression by immunohistochemistry is increased in kidneys of patients with ADPKD when compared with normal kidneys [7]. Increased TGF-α mRNA expression as demonstrated by Northern analysis is seen in cyst-lining epithelial cells derived from kidneys of patients with ADPKD [8]. Mice transgenic for TGF-α develop renal cysts [9]. TGF-α is present in mitogenic quantities in cyst fluid from *bpk* mice (a murine model of ARPKD), and immunoprecipitation of TGF-α reduces this mitogenic effect (abstract; Sweeney et al, *J Am Soc Nephrol* 7:1610, 1996).

Transforming growth factor-α is produced as a prepropeptide that undergoes further processing at the cell membrane to produce the secreted moiety [reviewed in 10]. The shedding of the secreted moiety is mediated by tumor necrosis factor-α (TNF-α) converting enzyme (TACE). This metalloproteinase (also called ADAM 17) was initially recognized as the mediator of TNF-α shedding. However, studies of TACE knockout mice revealed a phenotype similar to TGF-α knockout mice and confirmed that these animals had impaired TGF-α secretion [11].

Key words: epidermal growth factor receptor ligand, transforming growth factor-α, autosomal-recessive PKD, proximal tubule cysts, collecting tubule cysts.

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We recently demonstrated that interruption of EGFR activity by an irreversible inhibitor of receptor tyrosine kinase activity produced a marked reduction in disease severity in murine ARPKD [12]. The current study (*1*) characterizes kidney TGF-α expression in the *bpk* model of ARPKD and (*2*) examines the therapeutic potential of blocking TGF-α bioavailability in ARPKD using a novel inhibitor of TACE.

METHODS

The *bpk* model of ARPKD

This model arose from a spontaneous mutation in a colony of BALB/C mice. Affected animals have many similarities to the human disease including collecting tubule (CT) cysts and biliary ectasia and fibrosis [13]. The kidney disease has a consistent and severe phenotype. Mice homozygous for the *bpk* mutation have microscopic evidence of cyst formation at birth. Proximal tubule (PT) cysts are present at birth, which are gradually replaced by CT cysts as the disease progresses. Cyst expansion and kidney fibrosis result in death due to renal failure at 24 to 28 days. Heterozygotes show no phenotypic abnormalities and are identified by their ability to breed affected offspring. Unaffected (noncystic) littermates of cystic *bpk* mice are either wild-type or heterozygous at the *bpk* locus.

Pharmacology of WTACE2 and WAR-1

WTACE2 is a novel metalloproteinase inhibitor specific for TACE that was developed by Wyeth-Ayerst Research (Pearl River, NJ, USA). WTACE2 is a nonpeptide sulfonamide TACE inhibitor bearing a hydroxamic acid as the requisite zinc-chelating moiety. This nanomolar level inhibitor contains a novel butynyloxybenzene P1′ group, derived from analysis of the TACE x-ray structure, which provides enhanced cellular activity. WTACE2 is an orally bioavailable, broad-spectrum inhibitor of matrix metalloproteins (MMPs) with low nanomolar activity in vitro against MMP-1, MMP-9, and MMP-13. WAR-1 is a global matrix metalloproteinase inhibitor that is a potent inhibitor of MMP-9 and MMP-13, with no measurable activity against TACE.

TGF-α expression in *bpk* mice

Kidneys were obtained from cystic *bpk* mice and noncystic littermates at postnatal days 7, 14, and 21. Immunohistology was performed with modifications as described previously on formaldehyde-fixed specimens embedded in plastic [12]. Primary antibody was a polyclonal anti-TGF-α (Chemicon, Temecula, CA, USA) directed against recombinant 6 kD human TGF-α and reactive to mouse. Tubular localization of antibody staining was assessed by staining of serial sections with segment-specific biotinylated lectins, as described [12, 14]. All reagents for these and subsequent experiments were obtained from Sigma (St. Louis, MO, USA), unless indicated.

Protein was isolated from whole kidneys by homogenization in RIPA buffer (phosphate-buffered saline containing 1% nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS) with inhibitors [0.1 mg/mL aprotinin, 5 μg/mL leupeptin, 50 μg/mL pepstatin, 1 mmol/L ethylenediaminetetraacetic acid (EDTA), 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), and 1:100 vol/vol phosphatase inhibitor cocktail]. Protein content of all samples was determined using the BCA protein assay kit (Pierce, Rockford, IL, USA), and equal loading was confirmed by Ponseau S solution staining of membranes following transfer.

For Western blotting, 30 μg of total protein lysate was diluted in SDS reducing buffer [62.5 mmol/L Tris-HCl, pH 6.8, 25% vol/vol glycerol, 2% wt/vol sodium dodecyl sulfate (SDS), 0.01% wt/vol bromophenol blue, 5% vol/vol β-mercaptoethanol] and subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using a 12% separating gel. Samples were transferred to a nitrocellulose membrane, hybridized with blocking buffer (5% dry milk, 0.05% Tween 20) and then hybridized with mouse monoclonal anti-TGF-α (Research Diagnostics, Flanders, NJ, USA). Membranes were washed and hybridized with peroxidase conjugated anti-mouse antibody. Membranes were treated with the enhanced chemiluminescence (ECL) reagent (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and exposed to autoradiography film.

Cyst fluid from day 21 *bpk* mice also was examined for the presence of TGF-α by immunoprecipitation; 200 μg of total cyst fluid protein was immunoprecipitated with 2 μg of primary antibody (polyclonal anti-TGF-α; Santa Cruz, CA, USA). Then Protein A/G PLUS-agarose (Santa Cruz) added, and the incubation continued. Pellets were collected by centrifugation, washed, and resuspended in 1 × SDS reducing buffer and boiled for two to three minutes. Western analysis was then performed as described previously in this article.

WTACE2 treatment of *bpk* mice

Cystic *bpk* mice and phenotypically normal littermates were injected with a dose of 100 mg/kg/dose of WTACE2 given intraperitoneally once daily, in a vehicle containing 0.5% methocellulose (Fluka Biochemica, Ronkonkoma, NY, USA) and 2% Tween 80 (JT Baker, Phillipsburg, NJ, USA), beginning at postnatal day 7. Age-matched untreated cystic *bpk* mice and their noncystic littermates served as controls. An initial trial (data not shown) demonstrated that vehicle-injected cystic and noncystic mice had kidney weight to body weight ratios comparable to untreated mice. For the studies conducted in this trial, untreated cystic and noncystic mice served as controls. At day 21, mice were sacrificed. Blood was obtained by orbital puncture prior to sacrifice. Kidney weight and body weight for treated and untreated cystic and noncystic mice were

measured at sacrifice. Blood urea nitrogen (BUN) was assessed using a colorimetric assay. Serum creatinine was assessed using standard techniques in the hospital laboratory. Differences in clinical and laboratory parameters between treated and untreated cystic and noncystic mice were analyzed by the two-tailed Student *t* test.

Kidneys were fixed in 4% paraformaldehyde and embedded in plastic, as described [12]. Segment-specific localization of cysts was assessed using lectins specific to proximal tubule [*Lotus tetragonolobus* (LTA)] and collecting tubule [*Dolichos biflorus* agglutinin (DBA)]. Serial LTA and DBA stained sections were examined by light microscopy and assessed for severity of cystic dilatations in PTs and CTs, expressed on a scale of 0 to 5 using a modified cystic index [12]: 0 = no cysts; 1 = ≤0.11 mm; 2 = 0.12 to 0.19 mm; 3 = 0.20 to 0.27 mm; 4 = 0.28 to 0.35 mm; and 5 = ≥0.36 mm. The total number of CT (DBA+/LTA−) cysts and PT (LTA+/DBA−) cysts within a section were counted and expressed as a ratio.

In order to determine whether inhibition of secreted TGF-α affected total kidney expression of TGF-α protein, TGF-α expression in WTACE2-treated and untreated cystic and noncystic animals was assessed by Western analysis as described previously in this article.

Treatment with WAR-1, an MMP inhibitor without TACE activity

To determine whether a treatment effect of WTACE2 could be due to its activity against MMPs other than TACE, a limited treatment trial with WAR-1, a novel MMP inhibitor without TACE activity, was undertaken. Two litters of *bpk* mice and their noncystic littermates were treated with dosages of 50 mg/kg/day given as once daily intraperitoneal injections. This dose was the highest dose that could be given without significant mortality. Mice were treated from day 7 of life until day 21 and then sacrificed as described previously in this article. Analysis of WAR-1–treated mice included assessment of kidney weight to body weight ratio.

RESULTS

Kidney TGF-α expression in *bpk* mice

In the early stage of disease (day 7), immunohistology demonstrated markedly increased TGF-α expression in PT (LTA+/DBA−) cysts. As shown in Figure 1a, TGF-α expression in PTs localized to both the apical and basolateral membranes, with staining particularly prominent along the apical membrane. Low levels of TGF-α staining were seen in the PTs of age-matched non-cystic littermates. However, the intensity of staining was much lower and minimal apical staining was present (Fig. 1b). With advanced disease in the *bpk* mouse, CT cysts predominate. As shown in Figure 1c, TGF-α expression with apical staining was seen in CT cysts (DBA+/LTA−). In the remaining PT cysts, intense apical expression persisted. Cyst fluid staining positive for TGF-α also was seen in the lumens of several cysts at advanced stages of the disease. The tubular origin of these very large cysts could not be determined by lectin staining. However, failure of lectin expression in larger cysts is not uncommon in the advanced stages of disease (unpublished observations) and may reflect deterioration of the epithelium with loss of normal segment-specific markers. Lower levels of TGF-α expression were seen in PTs and CTs of noncystic littermates, again at a much lower intensity and with minimal apical expression (Fig. 1d).

Figure 2 demonstrates TGF-α expression by Western analysis of whole kidney lysates at days 7, 14, and 21 (early, middle, and late stages of disease) in cystic *bpk* mice and noncystic littermates. Early in the disease course, total TGF-α levels were similar. However, with progressive disease, there was a marked increased in TGF-α expression in cystic kidneys compared with noncystic kidneys. TGF-α was present in cyst fluid obtained from day 21 *bpk* mice, as evaluated by immunoprecipitation. Dominant bands in the whole kidney lysates were noted at 30, 34, and 61 kD and in cyst fluid at 6, 30, and 61 kD.

In vivo trials of WTACE2

A total of 6 cystic *bpk* mice and 27 noncystic littermates were treated with WTACE2. Fifteen age-matched cystic *bpk* mice and 30 noncystic littermates served as untreated controls. Cystic *bpk* mice treated with WTACE2 showed a significant 43% reduction in kidney weight to body weight ratio when compared with untreated cystic mice (Table 1). In parallel, kidney weights of treated cystic mice decreased significantly (50% reduction) when compared with untreated cystic mice (0.93 vs. 1.83 g). The body weights of treated and untreated cystic mice did not differ significantly. The treated and untreated noncystic mice showed no differences in kidney weights, body weights, or kidney weight to body weight ratios. These data, as well as biochemical parameters of treated and untreated cystic and noncystic mice, are summarized in Table 1. The gross appearance and relative sizes of representative kidneys from each treatment group are illustrated in Figure 3. Comparisons of renal function between treated and untreated cystic mice demonstrated a 30% improvement in BUN (33 vs. 50 mg/dL). A similar improvement in serum creatinine values (0.18 vs. 0.28 mg/dL) was seen but did not reach statistical significance. There were no differences in renal function parameters between the two noncystic groups (Table 1).

Daily injections of WTACE2 were generally well tolerated. All treated mice survived to sacrifice except one mouse that developed diarrhea at day 18 and died at day 20. One additional animal developed diarrhea but

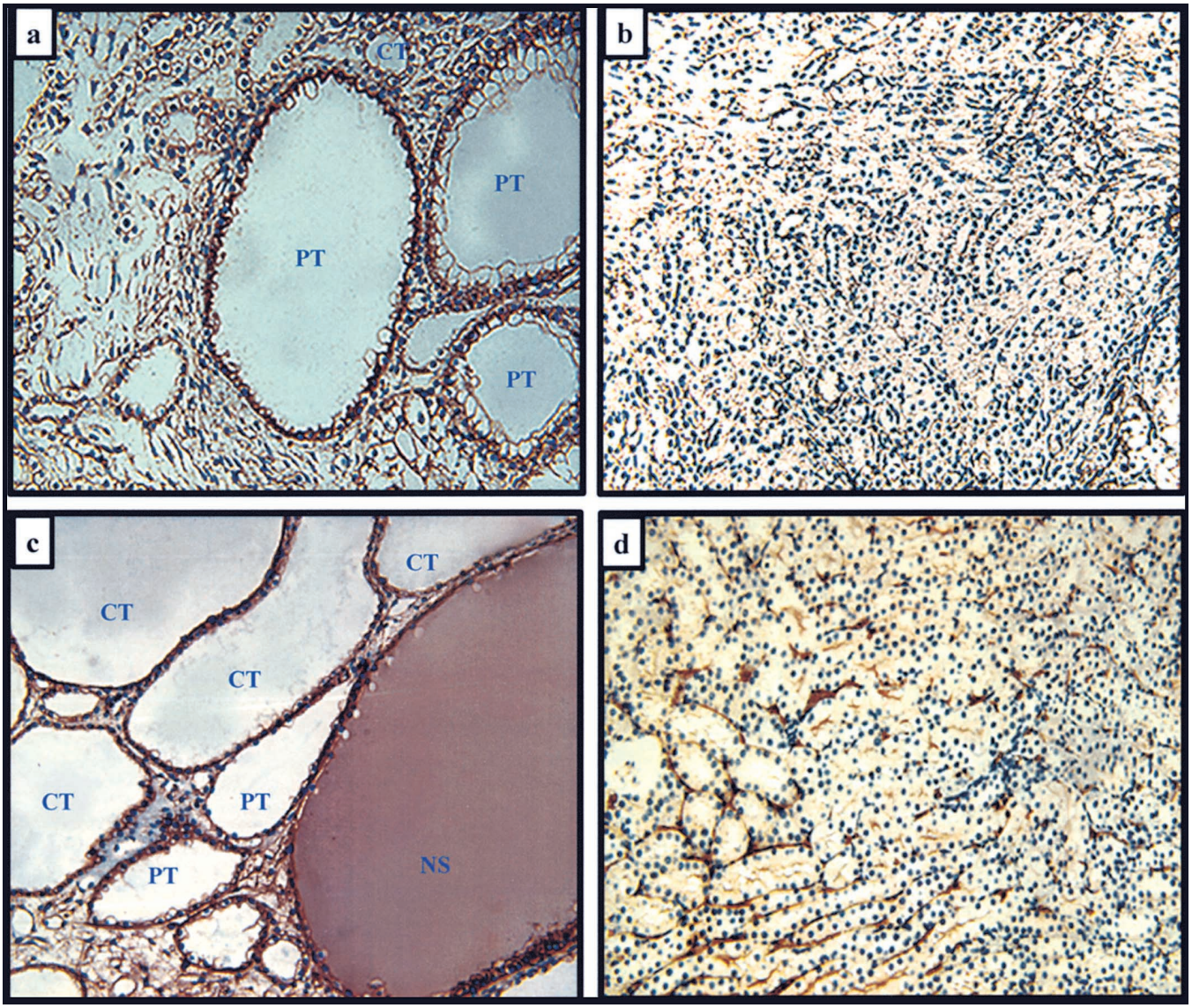

Fig. 1. Transforming growth factor-α (TGF-α) expression by immunohistology in *bpk* kidneys and age-matched noncystic mice at early and late stages of disease. (*a*) Immunohistology with polyclonal TGF-α (brown-staining) shows intense staining of proximal tubule (PT) cysts (LTA+/DBA−) at day 7, with apical staining particularly prominent. Occasional small collecting tubule (CT) cysts (DBA+/LTA−) also show staining. (*b*) Day 7 noncystic kidney has low levels of TGF-α staining in the PTs, primarily in the basolateral distribution with minimal apical expression. (*c*) As the disease progresses, TGF-α expression increases and is present in both CT cysts as well as residual PT cysts, with prominent apical and basolateral expression, as shown in this day 21 cystic kidney. Large cysts are occasionally seen with peroxidase positive material in the lumen. The tubular origin of these cysts could not be determined, but are presumed to be CT, as noted in the text (NS = nonstaining for LTA or DBA). (*d*) Noncystic day 21 kidney with some TGF-α staining evident in the CTs, in a basolateral distribution. Minimal apical staining is evident in CTs or PTs (original magnification ×20).

recovered without apparent ill effects. No other apparent toxicities were observed.

Histologic characteristics of treated and untreated cystic mice are summarized in Table 2. Treatment with WTACE2 was associated with an improvement in cystic CT lesions, as illustrated in Figure 4. The mean CT cystic index of treated cystic mice was 35% less than that of untreated cystic mice (3.2 vs. 4.8). Maximal cyst size in the treated cystic mice was 0.29 mm compared with a maximum of 0.41 mm in the untreated cystic mice, paralleling the overall reduction in kidney weight to body weight ratio. The mean PT cystic index in cystic treated mice was slightly greater than that of the untreated groups (1.8 vs. 1.4); however, this difference did not reach statistical significance. The ratio of cystic CTs to PTs was diminished in treated compared with untreated cystic mice (1.2 vs. 8.0), and the values obtained were similar to those of untreated mutant mice at an earlier stage of disease [13].

Western analysis of kidneys of treated and untreated

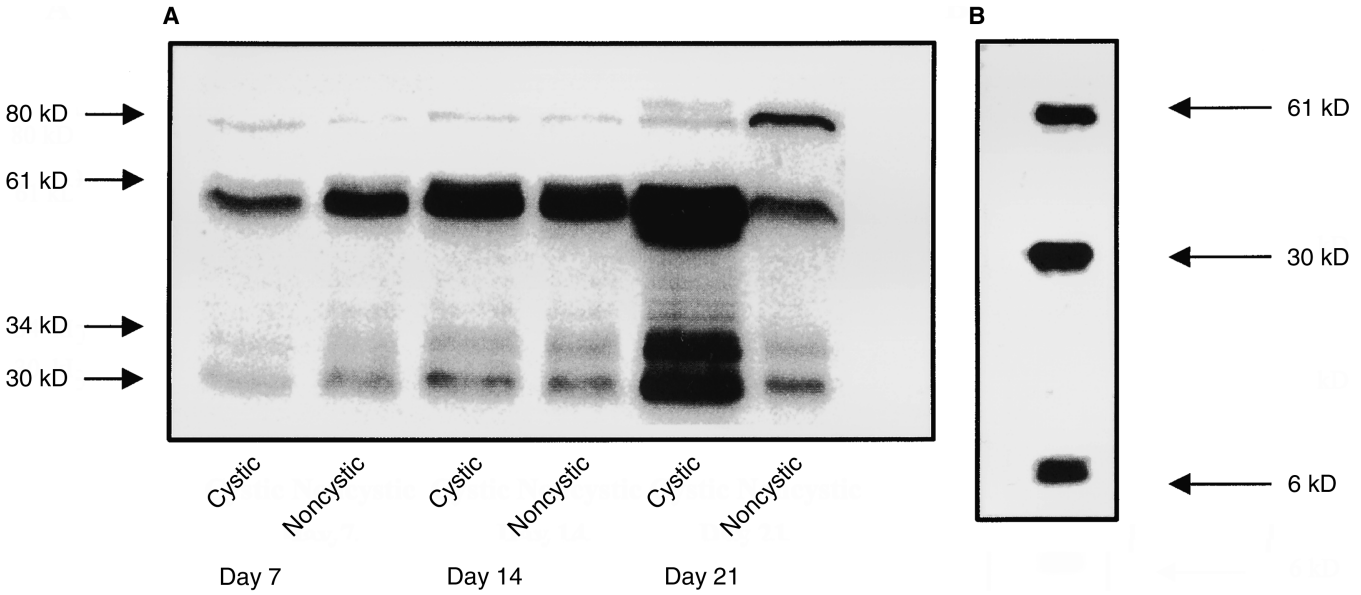

Fig. 2. TGF-α protein expression by Western analysis in early, middle and late disease in bpk mice and noncystic littermates. (*A*) Western analysis of whole kidney lysates at day 7, 14, and 21 in cystic *bpk* mice and noncystic littermates was performed as outlined in the text. TGF-α expression is increased in cystic kidneys compared to noncystic kidneys as disease progresses. Prominent bands are present at 30, 34, and 61 kD in kidney lysates. (*B*) Immunoprecipitation of cyst fluid from day 21 cystic *bpk* mice was performed as described in the text. The mature secreted 6 kD species is evident; bands at 30 and 61 kD are also present.

Table 1. Clinical parameters of WTACE2-treated and untreated mice

Treatment group	Kidney weight	Body weight	Kidney weight to body weight	BUN	Creatinine
	g		%	*mg/dL*	
P-21 Cystic ($N=15$)	1.83 ± 0.6	9.3 ± 2.2	19.7 ± 3.4	50 ± 9[a]	0.28 ± 0.13[a]
P-21 Cystic + WTACE2 ($N=6$)	0.93 ± 0.2[f]	8.2 ± 1.3	11.2 ± 1.3[f]	33 ± 4[bf]	0.18 ± 0.05[b]
P-21 Noncystic ($N=30$)	0.13 ± 0.01	9.1 ± 1.0	1.5 ± 0.1	19 ± 4[c]	0.14 ± 0.07[e]
P-21 Noncystic + WTACE2 ($N=27$)	0.13 ± 0.01	8.5 ± 1.0	1.5 ± 0.1	17 ± 4[d]	0.15 ± 0.05[e]

[a] $N = 5$; [b] $N = 4$; [c] $N = 12$; [d] $N = 14$; [e] $N = 10$; [f] $P < 0.01$ cystic treated compared to cystic untreated

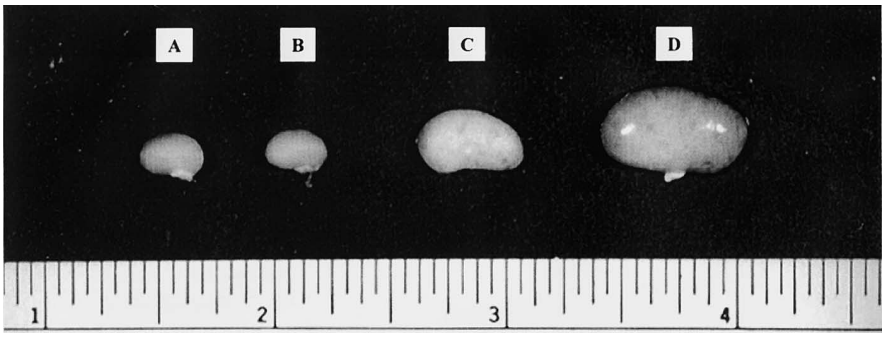

Fig. 3. Whole kidney micrograph from day 21 WTACE2-treated and untreated cystic *bpk* mice and noncystic littermates. Micrograph demonstrating relative kidney sizes of WTACE2-treated and untreated cystic bpk mice and noncystic littermates. (*A*) WTACE2-treated noncystic, (*B*) untreated noncystic, (*C*) WTACE2-treated cystic, and (*D*) untreated cystic. Kidneys were harvested at day 21. Cystic-treated mice have decreased kidney size compared to untreated cystic mice. Untreated and treated noncystic kidneys are not significantly different in size.

Table 2. Kidney histology of WTACE2-treated and untreated cystic mice

Treatment group	CT cystic index (graded 1–5)	CT cyst size *range mm*	PT cystic index (graded 1–5)	PT cyst size *range mm*	Cystic CT/PT ratio
Cystic no treatment ($N=5$)	4.8 ± 0.4	0.012–0.41	1.4 ± 0.5	0.012–0.13	8
Cystic + WTACE2 ($N=5$)	3.2 ± 0.4[a]	0.012–0.29	1.8 ± 0.4	0.012–0.17	1.2[a]

[a] $P < 0.01$ cystic treated compared to cystic untreated

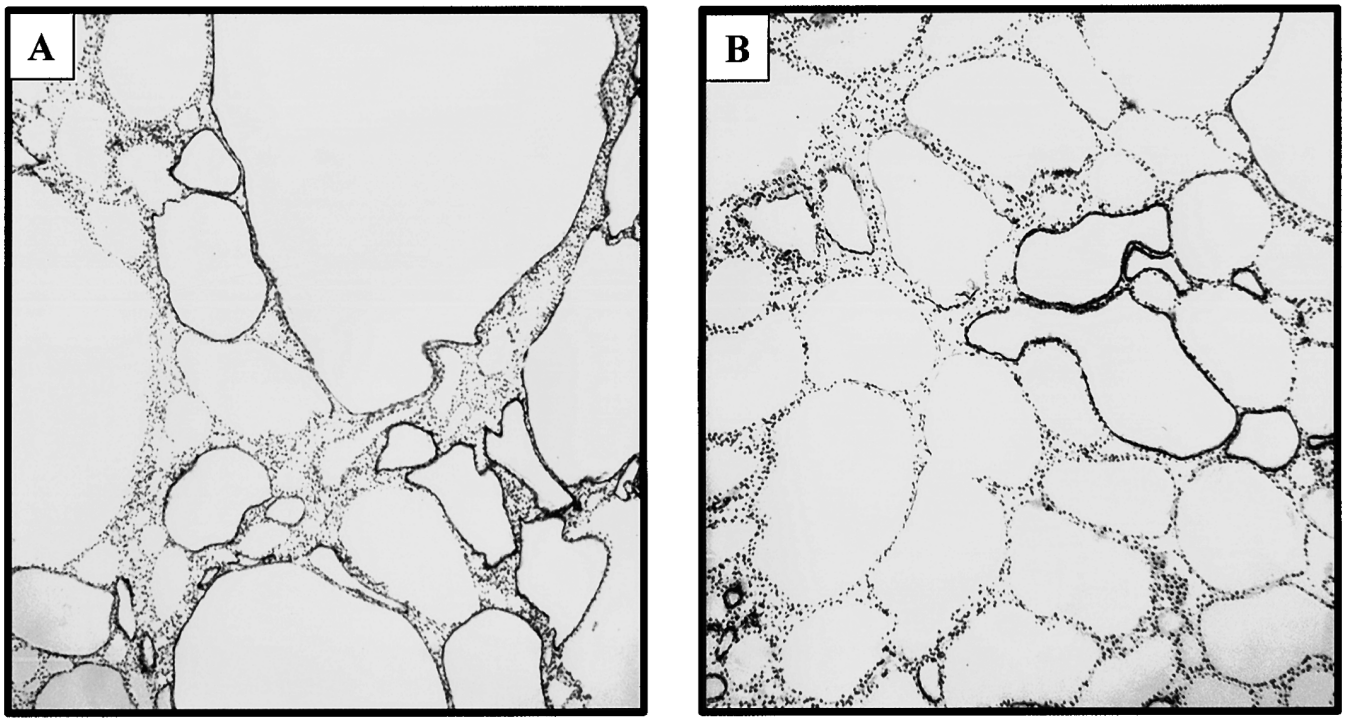

Fig. 4. Histologic appearance of cystic lesions in WTACE-treated and untreated day 21 *bpk* mice. Lectin binding of day 21 *bpk* mice (*A*) untreated and (*B*) treated with WTACE2. Immunohistology with biotin-labeled DBA (DBA+ = browning staining) shows a decrease in the size of CT cysts as well as a decrease in the relative number of CT cysts. Remaining cysts stained positive for LTA (proximal tubule) on serial sections. A small percentage of larger cysts were neither DBA nor LTA positive.

cystic and noncystic mice demonstrated a reduction in overall TGF-α expression in treated cystic mice compared with untreated cystic mice (Fig. 5). There were no differences in TGF-α expression in the two noncystic groups.

A total of 3 cystic *bpk* mice and 12 noncystic littermates were treated with WAR-1, a global MMP inhibitor without in vitro activity against TACE (Table 3). The "relative activity" values shown in Table 3 were derived from the inverse of the inhibitory concentration 50 (IC50) and expressed on a scale of 1 to 10 (1, less active; 10, very active). A higher number, therefore, indicates greater in vitro inhibition. Treatment of cystic *bpk* mice with WAR-1 caused a 28% reduction in the kidney weight to body weight ratio compared with untreated cystic mice. The reduction in kidney weight to body weight ratio was less than that of cystic animals treated with WTACE2 (Table 4).

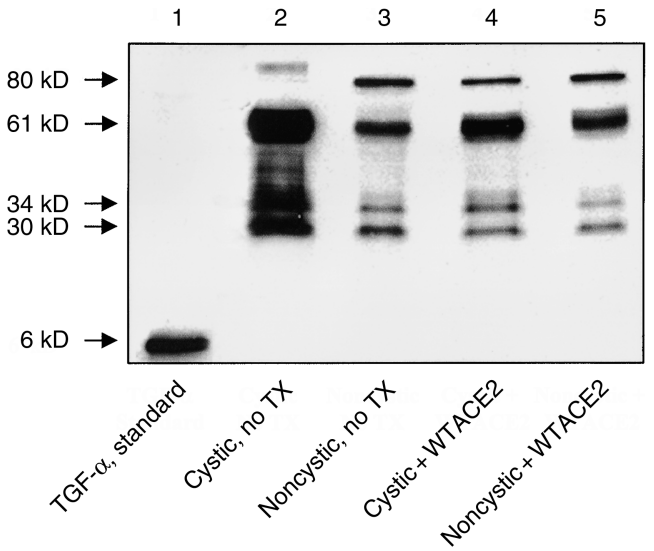

Fig. 5. TGF-α expression in kidneys of WTACE2-treated and untreated day 21 cystic *bpk* mice and noncystic littermates. Western blotting was performed on whole kidney homogenates as described in the text. Recombinant human TGF-α (molecular weight = 6 kD) was loaded in lane 1 as a reference. Kidneys of cystic mice treated with WTACE2 (lane 4) have a reduction in overall TGF-α protein expression compared with untreated cystic mice (lane 2). There are no differences in TGF-α expression in untreated and treated noncystic kidneys (lanes 3 and 5). No TX = no treatment.

DISCUSSION

This study delineates both quantitative and qualitative abnormalities in TGF-α expression throughout the disease course in *bpk* mice. Prominent TGF-α staining present on the apical surface of PTs early in the disease course suggests a primary abnormality in TGF-α expression early in disease. This finding further suggests that PTs may

Table 3. Relative in vitro activity[a] of WTACE2 versus WAR-1 against MMPs 1, 9, 13 and TACE

Treatment	MMP-1	MMP-9	MMP-13	TACE
WTACE 2	1.3	0.7	3	1.1
WAR-1	0	8.2	10	0

Abbreviations are: WTACE2, competitive inhibitor of tumor necrosis factor-α converting enzyme; WAR, global matrix metalloprotein; MMP, matrix metalloprotein; TACE, tumor necrosis factor-α converting enzyme.

[a] Expressed on a scale of 1-10 (less active-more active)

Table 4. Treatment effect on cystic kidney disease of WTACE2 versus WAR-1

Treatment	Cystic kidney weight to body weight *percent*
WTACE2 ($N = 6$)	11.2 ± 1.3
WAR-1 ($N = 3$)	15.3 ± 1.7
No treatment ($N = 15$)	19.7 ± 3.4

serve as a large source for secreted ligand upstream from CTs. This raises the possibility of paracrine activation of EGFR at the earliest stages of disease, which may promote CT cell proliferation and cyst development. With advanced disease, TGF-α is present on the apical (and basolateral) surfaces of both CTs and PTs, suggesting a mechanism by which both paracrine and autocrine activation of EGFR might occur later in the disease course.

Western analysis demonstrated a progressive increase in TGF-α expression in whole kidney lysates from cystic mice. Several different protein species were evident on the blots (Figs. 3 and 5). The 34 kD band was present only in whole kidney lysates but was absent from the cyst fluid IP. This suggests that the 34 kD band may be a membrane-bound form. In contrast, the 6 kD form, corresponding to the mature, secreted moiety was evident in cyst fluid but not seen in whole lysates. It therefore appears that the relative amount of mature secreted ligand, in comparison to total membrane-bound ligands (or higher molecular weight secreted forms), was insufficient to be detected except in the "enriched" source, that is, cyst fluid. The remaining bands may represent either alternatively processed secreted forms (since they are present in both the whole kidney lysates and the cyst fluid) or nonspecific findings. A variety of TGF-α species, either secreted or membrane-bound, ranging in size from 6 to 60 kD, have been reported in normal and malignant cells and tissues [15–17].

WTACE2 is a potent inhibitor of TACE, the enzyme that mediates cleavage of TGF-α to release the secreted moiety. This study demonstrates that inhibition of TGF-α secretion can slow disease progression in mice with ARPKD. Treated mice had a significant reduction in kidney size as well as improvement in renal function. A reduction in the CT cystic index in treated mice suggests that the reduction in kidney size was due to an overall decrease in the size of the cystic lesions. The most likely explanation for this decrease in cyst size is that reduced ligand availability resulted in reduced tubular epithelial cell proliferation. Since cystic CTs express functional apical EGFR [3, 6], the reduction of biologically active ligand secreted into the lumen is likely to decrease EGFR-mediated cell proliferation. We therefore hypothesize that the decrease in cyst size in mice treated with WTACE2 is due to a decrease in the proliferative ability of cystic epithelium.

An alternative hypothesis for the therapeutic effectiveness of WTACE2 in vivo is alteration of MMPs other than TACE. Although WTACE2 has specific activity against TACE, it is also some degree of in vitro activity against MMP-1, MMP-9, and MMP-13 (Table 3). MMPs play an important role in remodeling extracellular matrix, which has been hypothesized as a necessary step for cyst expansion [18]. Recently, a global metalloproteinase inhibitor, batimastat, with activity against MMPs, was studied in a rat model of ADPKD. Rats treated with batimastat for eight weeks showed a reduction in cyst number and kidney weight (abstract; Obermuller et al, *Fifth International Workshop on PKD*, 2000). Treatment of cystic mice with WAR-1, an MMP inhibitor with no activity against TACE, also produced a reduction in kidney weight to body weight ratio, although this was less than the reduction seen with WTACE2 treatment (28% compared with 43% with WTACE2). In vitro profiles of WTACE2 and WAR-1 demonstrate that they both share some degree of activity against MMP-9 and MMP13, although the activity of WTACE2 is considerably lower than that of WAR-1 (Table 3). Although the number of cystic mice treated with WAR-1 was small, the findings suggest the possibility that the therapeutic benefit of WTACE2 could be due, in part, to inhibition of one or both of these compounds.

Epidermal growth factor receptor (EGFR) ligands such as TGF-α may directly alter expression of certain metalloproteinases, including MMP-9, in cells that overexpress EGFR [19, 20]. Both MMP-2 and MMP-9 are up-regulated in murine ARPKD [18, 21]. Principal cells, the primary cells affected in ARPKD, express both MMP2 and MMP9 [22]. Furthermore, MMP9 expression in these cells is up-regulated by treatment with EGF, another EGFR ligand [22]. By inhibiting shedding of TGF-α, WTACE2 may impact indirectly on those pathways as well. These potential mechanisms for inhibiting cyst growth are not mutually exclusive, and it is possible that both are operative in mediating the therapeutic effectiveness of WTACE2.

Tumor necrosis factor-α converting enzyme mediates

TGF-α shedding, but based on its mode of action, it would not be expected to affect overall expression levels of the protein. However, when TGF-α expression was examined in kidney lysates of animals treated with the TACE inhibitor WTACE2, the expression levels of treated cystic mice were considerably lower than those of untreated cystic mice (Fig. 5). Kidney TGF-α levels of treated and untreated noncystic mice were comparable and lower than those of treated or untreated cystic mice. Published reports of malignant and normal cell lines have shown that EGFR ligands may up-regulate their own production (auto-induction) or that of other EGFR ligands (cross-induction) [23–25]. We hypothesize that by decreasing the availability of secreted ligand, WTACE2 may inhibit these auto-inductive or cross-inductive phenomena. Kidney TGF-α expression in cystic treated mice was still greater than that of noncystic mice, confirming that the aberrant TGF-α expression seen in cystic mice was not completely eliminated.

WTACE2 was well tolerated in vivo, and there were no apparent toxicities. In particular, there were no untoward effects on growth. Mouse weight, kidney weight, and kidney weight/body weight ratios were comparable in treated and untreated noncystic mice. The findings of comparable growth parameters in the treated and untreated noncystic mice suggest that the effects of WTACE2 were not due to a global inhibition of growth. In the batimastat trial discussed previously in this article, fibrotic capsules were noted around the kidneys of some treated animals, which could have contributed to the decrease in kidney size reported (abstract; Obermuller et al, *Fifth International Workshop on PKD*, 2000). In contrast, animals treated with WTACE2 showed no such abnormalities.

The effects of WTACE2 on cystic disease progression in *bpk* mice were not as striking as the effects of the EGFR tyrosine kinase inhibitor, EKI-785 [12, 26]. There are a number of possible explanations for these differences. WTACE2 inhibits production of the secreted moiety of TGF-α but has no known activity against membrane-bound forms. In vitro studies in other disease processes characterized by EGFR overexpression have suggested that membrane-bound forms of the ligand may be able to activate EGFR without the need for processing to the secreted form [27–29]. The relative pathophysiological importance of membrane-bound versus secreted forms of TGF-α in activating EGFR has not yet been studied in PKD. Immunohistology demonstrated that TGF-α was present on the apical surface of PTs and CTs in the later stages of disease. This finding suggests that activation of apical EGFR on CT cysts could occur by a paracrine (secreted TGF-α from PTs or CTs) or an autocrine (membrane-bound TGF-α on CTs) mechanism. Inhibition of TACE processing and secretion with WTACE2 would have little effect on the autocrine pathway.

An additional explanation for differential effects of TACE and EGFR tyrosine kinase inhibition relates to the complex biology of EGFR ligands. Multiple EGFR ligands are capable of binding and activating EGFR [30, 31]. In addition to EGF and TGF-α, which have been studied in PKD, other ligands that have not been studied to date include amphiregulin, heparin-binding EGF, betacellulin, and epiregulin. Preliminary unpublished data from our lab suggest that amphiregulin expression may be aberrant in PKD (abstract; Dell et al, *J Am Soc Nephrol* 11:388, 2000). At present, there are no published data to indicate whether TACE mediates shedding of the other EGFR ligands. Batimastat, a global MMP inhibitor, inhibits EGF and AR as well as TGF-α in vitro [29]. As noted previously in this article, treatment with batimastat in a rat model of ADPKD was associated with decreased cyst size and kidney size. However, this treatment did not effectively inhibit disease progression.

In conclusion, this study demonstrates that (*1*) quantitative and qualitative abnormalities in TGF-α expression are present in murine ARPKD and (*2*) treatment with an inhibitor of TGF-α secretion slowed the progression of renal disease in murine ARPKD. These findings provide additional support for the hypothesis that EGFR ligand up-regulation and resultant activation of EGFR contributes to the pathogenesis of ARPKD. They further suggest that TGF-α may have a particularly important role in this process. These findings suggest that a number of possible therapeutic options may emerge from a better understanding of the complex interrelationships between the EGFR ligands and the various pathways upon which they act in PKD. The findings of this study and our previous work also raise the possibility that combinations of therapies directed at EGFR and one or more of its ligands may be of additional clinical benefit in the treatment of PKD.

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