The Raf kinase inhibitor PLX5568 slows cyst proliferation in rat polycystic kidney disease but promotes renal and hepatic fibrosis

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Abstract

Background. Autosomal dominant polycystic kidney disease (ADPKD) is a common cause of renal failure. Aberrant epithelial cell proliferation is a major cause of progressive cyst enlargement in ADPKD. Since activation of the Ras/Raf signaling system has been detected in cyst-lining epithelia, inhibition of Raf kinase has been proposed as an approach to retard the progression of ADPKD.

Methods and results. PLX5568, a novel selective small molecule inhibitor of Raf kinases, attenuated proliferation of human ADPKD cyst epithelial cells. It reduced in vitro cyst growth of Madin–Darby Canine Kidney cells and of human ADPKD cells within a collagen gel. In male cy/+ rats with polycystic kidneys, PLX5568 inhibited renal cyst growth along with a significant reduction in the number of proliferating cell nuclear antigen- and phosphorylated extracellular signal-regulated kinase-positive cyst-lining epithelial cells. Furthermore, treated animals showed increased capacity to concentrate urine. However, PLX5568 did not lead to a consistent improvement of renal function. Moreover, although relative cyst volume was decreased, total kidney-to-body weight ratio was not significantly reduced by PLX5568. Further analyses revealed a 2-fold increase of cellular signal-regulated kinase-positive cyst-lining epithelial cells. Furthermore, treated animals showed increased capacity to concentrate urine. However, PLX5568 did not lead to a consistent improvement of renal function. Moreover, although relative cyst volume was decreased, total kidney-to-body weight ratio was not significantly reduced by PLX5568. Further analyses revealed a 2-fold increase of cAMP-dependent activation of ERK and cell proliferation [15, 16]. Inhibition of MEK has been shown to slow the progression of PKD in pcy mice [17] but failed to affect PKD progression in mice with a kidney-specific knockout of PKD1 [18].

We therefore wondered if upstream inhibition of Raf kinases may reduce cell proliferation and attenuate cyst growth in ADPKD.

Conclusions. PLX5568 attenuated cyst enlargement in vitro and in a rat model of ADPKD without improving kidney function, presumably due to increased renal fibrosis. These data suggest that effective therapies for the treatment of ADPKD will need to target fibrosis as well as the growth of cysts.

Keywords: ERK; polycystic kidney disease; proliferation; Raf

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) accounts for ~10% of cases of chronic renal failure requiring renal replacement therapy [1, 2]. Cyst enlargement is driven by an imbalance of luminal secretion and absorption as well as by increased cyst cell proliferation [3, 4]. One of the driving forces for cyst cell proliferation is the induction of the Ras/Raf signaling cascade activated by insulin-like growth factor 1 [5], Src kinase [6] and accumulation of cyclic AMP (cAMP) in concert with Ca2+ dysregulation [7, 8].

Raf kinases also known as MAPKKK are part of the mitogen-activated protein kinase (MAPK) cascade activating the MAPK–ERK kinase MEK, which then activates ERK via phosphorylation [phosphorylated extracellular signal-regulated kinase (P-ERK)] of a threonine and a tyrosine residue. P-ERK translocates to the nucleus where it regulates the activities of various transcription factors [9–11].

In the kidney, the mainly C-Raf is found in tubular cells where it is involved in many physiological functions [12–14]. In polycystic kidney disease (PKD) also B-Raf, normally quiescent in kidney tubule cells, is upregulated and appears to be essential for cAMP-dependent activation of ERK and cell proliferation [15, 16]. Inhibition of MEK has been shown to slow the progression of PKD in pcy mice [17] but failed to affect PKD progression in mice with a kidney-specific knockout of PKD1 [18]. We therefore wondered if upstream inhibition of Raf kinases may reduce cell proliferation and attenuate cyst growth in ADPKD.

Materials and methods

Animals and treatment

A Sprague–Dawley rat model of PKD (Han:SPRD), with animals obtained from a breeding colony from Dr F. Deerberg (central institute for laboratory animal breeding, Hannover, Germany) and maintained by N.G., was used. Since 1997, the strain is registered in the list of inbred strains of rats by MFW Festing as PKD/Mhm. Experiments were approved by the institutional review board for the care of animal subjects and performed in accordance with National Institutes of Health guidelines. Animals had free access to tap water and standard rat chow. The light cycle was 12 h, humidity 55% and room temperature 20°C. After weaning, 28–30 days old male heterozygous (cy/+ ) rats were treated daily with PLX5568 (50 mg/kg, n = 7) or its vehicle (corn oil, n = 8) for 6 weeks (5–11 weeks of age) by oral gavage. Blood samples were collected...
were drawn at 0, 2, 4 and 6 weeks of treatment and urine was collected for 24 h. Rats were anesthetized with isoflurane before sacrifice.

**PLX5568**

PLX5568 was obtained from Plexikon, Berkeley. For in vitro assays, PLX5568 was dissolved in dimethyl sulfoxide (final solvent concentration <0.1%). For in vivo treatment, PLX5568 was suspended in corn oil. Effects of PLX5568 on in vitro Raf kinase activities were determined by measuring phosphorylation of biotinylated-MEK protein using AlphaScreen Technology (Perkin-Elmer). In addition, 225 selected kinases were profiled for inhibition by PLX5568 using the Z'-LYTE™ biochemical assay format (SelectScreen; Invitrogen) according to the manufacturer’s protocol.

**Immunohistochemistry**

Left kidneys were fixed with 3% paraformaldehyde (pH 7.4). For detection of proliferating cell nuclear antigen (PCNA), monoclonal mouse antibody (1:50; DAKO, Hamburg, Germany) and a biotinylated secondary anti-mouse antibody (1:200; Vector, Peterborough, England) were used. For detection of active Caspase 3, monoclonal rabbit antibody (1:100; Epitomics, Burlingame, CA) and a biotinylated secondary anti-rabbit antibody (1:200; Vector) were used. For detection of fibrosis, the following antibodies were used: mouse anti-fibronectin (1:500; Chemicon, Billerica, MA) at the Wisconsin MMP2 (1:50; Santa Cruz Biotechnology Inc., Santa Cruz, CA), goat anti-PAI 1 (1:50; American diagnostica GmbH, Pfungstadt, Germany), rabbit anti-FSP1 (1:100; Abcam, Cambridge, UK). Monoclonal mouse anti-P-ERK1/2 antibody (1:10 000; Cell Signalling, Boston, MA) and a signal amplification system (DAKO) were used according to the manufacturer’s instructions. Signals were analyzed with a Leica DMR microscope (Leica Microsystems, Wetzlar, Germany) and photographed by means of a Nikon Digital Sight DS-UK system.

**Histomorphometric analyses**

Cystic index was obtained by examining paraffin sections stained with Hematoxylin–Eosin at ×100 magnification, marking all cysts within the whole cortical area of each kidney with EclipseNet software (Nikon). Total cyst area was divided by the total cortex area.

For PCNA, P-ERK and cleaved Caspase 3 score, all cysts of each kidney section with diameters between 100 and 250 μm were analyzed in order to prevent cyst size-dependent bias at ×400 magnification. PCNA, P-ERK and cleaved Caspase 3-positive cells per cyst were counted and cyst perimeter was determined by EclipseNet software for standardization.

Fibrosis score was obtained by staining kidney and liver sections with picrosirius red or for fibronectin, MMP2, PAI 1 or FSP1. Photos of 10 random-field images per section were taken at a ×200 magnification. A colored threshold was applied using MetaVue™ Software (Molecular Devices®, Silicon Valley, CA) at a level that separated cysts from non-cystic tissue and picrosirius red-MMP2-PAI 1-FSP1-positive tissue from background. In the kidney sections, fibrosis is expressed as a percentage of total non-cystic tissue to avoid a potential bias due to different cyst sizes. Processing of all stainings of all tissue sections was done at the same time to avoid staining variability.

For all morphological analysis, the investigator was blinded to the experimental condition.

**Immunoblotting**

Kidney protein extraction and blotting were performed as described previously [19]. Twenty micrograms of pooled cortical proteins were resolved on polyacrylamide gels. Proteins were transferred onto an Immobilon-P membrane (Millipore, Bedford, MA) and probed with anti-P-ERK antibody (Santa Cruz, Heidelberg, Germany) at a concentration of 1 μg/mL. Signals were detected with horseradish peroxidase-conjugated antibodies (DAKO) and enhanced chemiluminescence (SuperSignal West Dura Extended; Pierce, Rockford, IL). Membranes were stained with Amido black to verify equal protein loading and transfer.

**ADPKD cell proliferation assay and cystogenesis**

Primary cultures of ADPKD cells were generated by the PKD Biometric Core at the University of Kansas Medical Center as described [8,15]. Cell proliferation of ADPKD cells was determined as described [7,15]. Briefly, 4000 cells were seeded into 96-well plates in Dulbecco’s modified Eagle’s medium/F12 medium containing 1% fetal bovine serum (FBS) and 5 μg/mL insulin, 5 μg/mL transferrin, and 5 ng/mL sodium selenite. After 24 h, cells were placed in media containing 0.002% FBS and treated with control media or 100 μM 8-Br-cAMP in the presence or absence of PLX5568. Proliferation was assessed 72 h after treatment using the Promega Cell titer 96 MTT Assay as described previously [20]. For cystogenesis, ADPKD cells were cultured within non-collagenized collagen gels and stimulated with 5 ng/mL epidermal growth factor (EGF) and 5 μM forskolin as described previously [20]. After cyst formation was initiated, EGF was removed, and forskolin and 20 nM PLX5568 were added individually or together to the culture medium.

**Madin–Darby canine kidney (MDCK) cyst growth**

Madin–Darby canine kidney (MDCK) cells were grown at 37°C at 21% O2 and 50% CO2 and maintained in Dulbecco’s modified Eagle’s medium supplemented with 2 mM l-glutamine, 10% heat-inactivated fetal calf serum, 50 IU/mL penicillin and 50 μg/mL streptomycin. MDCK cells were trypsinized and resuspended as a single-cell suspension of 1–2 × 10³ cells/mL in Type I collagen solution (PoreCol, Leumuiden, The Netherlands) as previously described [21]. Four hundred microliters of the suspension were filled in 24-well plates with 3–6 wells per condition. After 3–4 days of growth, 10, 100 or 1000 nM of PLX5568 or 10 μM of U0126 (Merck, Nottingham, England) were added. Medium was changed every 2 days. After 6–8 days, two random visual fields of each well were analyzed with an Olympus CK40 microscope (Olympus Lifes Science Research GmbH, Munich, Germany) and photographed (×40 magnification) by means of a Leica DC-200 system (Leica Microsystems). Cyst diameters of all captured cysts that were nearly spherical (150–350 cysts per condition and single experimental procedure) were measured with Eclipse-Net software (Nikon Instruments, Inc.). Cyst volume was then estimated using the formula for the volume of a sphere, 4/3 π r³.

**Statistics**

All results are expressed as mean ± SEM. A P-value <0.05 was considered significant. Statistical analyses were performed using two-tailed unpaired Student’s-t-test. Q–Q plots were used to assess the normality of empirical distributions. The software used was Prism 5 for Windows, version 5.02.

**Results**

**PLX5568 is a selective Raf kinase inhibitor**

In a Scaffold-Based Drug Discovery™ platform developed by Plexikon, PLX5568 has been identified as selectively targeting a unique binding site of the Raf protein, potentially highly selective for Raf kinases. In vitro, Raf kinase activities determined by measuring phosphorylation of biotinylated MEK protein using AlphaScreen Technology revealed an IC₅₀ of 31 nM for recombinant C-Raf and 250 nM for recombinant B-Raf kinase, respectively (Table 1). Using further the Z’-LYTE™ biochemical assay format, 225 selected kinases were profiled for inhibition by PLX5568. Raf kinase selectivity was confirmed showing only proto-oncogene tyrosine-protein kinase YES and the tyrosine kinase FER being inhibited by concentrations comparable to those necessary to inhibit C-Raf.

**PLX5568 attenuated cell proliferation and cyst growth in vitro**

In vitro cyst growth of MDCK cells within a collagen matrix was significantly reduced by PLX5568 in a dose-dependent manner (Figure 1A). Ten micromolar of the MEK-inhibitor U0126 diminished MDCK cyst growth comparably to PLX5568 used at a concentration of 1 μM (Figure 1B). Furthermore, PLX5568 inhibited 8-Br-cAMP-mediated cell proliferation of human ADPKD cyst epithelial cells in a dose-dependent manner (Figure 1C). In addition, PLX5568 significantly inhibited forskolin-mediated cyst growth of human ADPKD cells within a collagen gel at a low nanomolar range.
PLX5568 reduced cyst cell proliferation and cyst enlargement in cy/+ rats

Fifteen heterozygous male cy/+ rats were treated daily with vehicle (ctrl, corn oil, n = 8) or PLX5568 (PLX, 50 mg/kg body weight, n = 7) for 6 weeks (from 5 to 11 weeks of age) by oral gavage. Treatment with PLX5568 significantly reduced the cystic index (Figure 2A) and the number of PCNA-positive cyst-lining epithelial cells (Figure 2B). Consistent with these data, a significant reduction of P-ERK-positive cyst cells was observed (Figure 2C) as well as a reduction of the amount of P-ERK protein upon treatment with PLX5568 (Figure 2D). Apoptosis is a relevant factor for cyst progression in cy/+ rats [22]. However, PLX5568 neither had an effect on the apoptosis rate of the cyst-lining epithelium (Figure 2E) nor the kidney tubule cells (Figure 2F).

PLX5568 did not ameliorate kidney function in cy/+ rats

No obvious toxic effects by PLX5568 could be observed and treated animals showed a similar increase in body

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Table 1. IC50 values [nM] of PLX5568 against selected kinases

<table>
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<th>Kinase</th>
<th>C-Raf</th>
<th>B-Raf</th>
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<th>FER</th>
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<tr>
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<td>250</td>
<td>170</td>
<td>230</td>
<td>560</td>
<td>930</td>
</tr>
<tr>
<td>IC50 values &gt;10 000 nM</td>
<td>175 additional kinases</td>
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The C-Raf and B-Raf assays were run in 100 μM ATP, while the other kinases were run in 10 μM ATP.

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Fig. 1. PLX5568 attenuated cell proliferation and cyst growth in vitro. (A) MDCK cells were seeded in collagen I gels for 3 days and then treated with 0, 10, 100 or 1000 nM of PLX5568 for 8 days. Morphology and sizes of ~1100–1200 cysts per condition of six individual experiments were obtained and summarized as means ± SEM. *P < 0.05. (B) MDCK cells were seeded in collagen I gels for 4 days and then treated with 1 μM of PLX5568 or 1 μM of UO126 for 6 days. Morphology and sizes of ~1300–1500 cysts per condition of three individual experiments were obtained and summarized as means ± SEM. *P < 0.05. (C) Cell proliferation of human ADPKD cyst cells was stimulated with 100 μM 8-Br-cAMP in the presence or absence of different concentrations of PLX5568 as specified. After 72 h, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide-assays were performed showing the means ± SEM of four individual experiments. *P < 0.05. (D) Human ADPKD cells gained from five individual kidneys were dispersed within collagen gels and media containing 5 μM forskolin to induce cyst formation for 3 days. Then medium was replaced, containing either 5 μM forskolin or 5 μM forskolin + 20 nM PLX5568 for an additional 5 days. Gels were fixed in 1% formalin and total surface area of cysts with diameters >100 μm was measured.
weight as controls (Figure 3A). Unexpectedly, although leading to a reduced cystic index, PLX5568 did not ameliorate the two kidney-to-body weight ratio (Figure 3B). Furthermore, PLX5568 did not reduce proteinuria (Figure 3C). Creatinine levels tended to be slightly lower in PLX5568-treated animals, with a significantly lower level at weeks 2 and 6 of treatment (Figure 3D). However, creatinine levels in general are barely increased at this point in cy/+ rats compared to wild-type [23]. Furthermore, blood urea nitrogen (BUN) levels that are already 2-fold increased in cy/+ at week 10 of age [23, 24] were identical with those of treated animals (Figure 3E). Urine concentrating ability, which is impaired very early in PKD [25], was significantly improved by treatment with PLX5568 (Figure 3F) consistent with trends toward reduced diuresis (Figure 3G) and less water intake (Figure 3H) in PLX5568-treated animals.

**PLX5568 promoted renal and hepatic fibrosis in cy/+ rats**

Due to the divergent effects on cyst sizes in PLX5568-treated cy/+ rats but similar kidney-to-body weight ratios, we wondered if PLX5568 might lead to increased renal fibrosis. The fibrosis score obtained after staining with Sirius red and standardized regarding to the different cyst sizes in treated and untreated kidneys was 2-fold lower in PLX5568-treated animals (Figure 3D).
increased by PLX5668 (Figure 4A). To further confirm these data, we stained for fibronectin as an extracellular matrix protein, which was also significantly increased in PLX5568-treated animals (Figure 4B). In addition, we examined the expression of pro-fibrotic proteins reflecting tubular affection. Expression of matrix metalloproteinase-2 (MMP2), plasminogen activator inhibitor-1 (PAI 1) and S100 calcium-binding protein A4 (FSP1) was markedly increased in animals treated with PLX5568 (Figure 4C–E). To determine, if the increase in matrix was organ specific we then also analyzed fibrosis scores in the liver and found that hepatic fibrosis was also significantly increased by treatment with PLX5568 (Figure 4F).

**Discussion**

There is a significant interest in retarding the progression of ADPKD. Many of the current efforts are directed toward inhibition of cell proliferation. However, limited specificity and selectivity of such approaches are usually a problem. Here, we show that PLX5568, a novel selective inhibitor of Raf kinase, attenuates cyst growth of MDCK and human ADPKD cells as well as cyst growth in cy/ cy rats, however, lacking significant impact on kidney function which might be explained by increased kidney fibrosis.

The MAPK pathway as a potential therapeutic target in PKD has already attracted significant interest. In vivo, vasopressin V2 receptor antagonists have been shown to reduce the activity of the ERK1/2 pathway by reducing intracellular cAMP levels [26, 27]. In PCK rats, elevated water intake to reduce the renal effects of Arginine vasopressin reduced renal ERK activity, the number of PCNA-positive cells and kidney volume [28]. Furthermore, inhibition of MEK in pcy mice also led to decreased cyst proliferation [17]. In addition, use of PLX5568 in a PKD1 orthologous mouse model led to a significant reduction of kidney-to-body weight ratio, cystic index, BUN and cyst cell proliferation (Nishio et al., ASN Renal Week 2009, SA-PO2396). In contrast, recent studies by Shibazaki et al. [18] present evidence that inhibition of MEK by UO126 in a PKD1 conditional knockout model of PKD failed to inhibit disease progression which challenges the significance of Raf kinases in all PKD models.

The essential physiological relevance and ubiquitous expression of the MAPK pathway turns it into a complex target. The many physiological activation mechanisms of the MAPK pathway include branching of the Drosophila tracheal system [29] and of murine salivary glands [30]. In addition, MAPK activation plays a crucial role in distal tubular and collecting duct cells in order to protect them against hyposmotic stress, probably via regulation of aquaporin 2 [12, 31, 32]. In vitro, tubulogenesis and spreading of renal epithelial cells are also highly dependent on P-ERK [14, 33, 34].
Our data suggest that PLX5568 may preserve tubular function along with reduced cyst sizes, as reflected by improved urine concentrating ability starting already at week 2 of treatment. Unfortunately, rats were too small to collect urine at the starting point so we cannot rule out an unlikely but possible bias due to different urine concentrating abilities at day 0 between the two groups. However, loss of concentrating ability is a typical feature of most PKD animal models and of human ADPKD in spite of elevated levels of circulating vasopressin, renal cAMP and renal expression of vasopressin V2 receptors and AQP2 messenger RNA [35, 36]. Recent data suggest that the concentrating defect in polycystic kidneys may be caused by persistent activation of RhoA due to ERK-dependent Rho-GTPase activated protein inactivation and phospholipase A2 activation leading to inefficient translocation of AQP2 to the apical membrane [36]. Therefore, inhibition of the ERK pathway may lead to improved concentrating ability. Nevertheless, taking into account the absence of effects on creatinine and BUN levels and the significant increase of renal and hepatic fibrosis, it seems unlikely that PLX5568 may have overall beneficial effects in this model and that the increase of fibrosis may even outweigh beneficial effects.

Interestingly, general toxicology studies in normal rats do not show evidence of liver or kidney fibrosis (Gideon Bollag unpublished observations). Untreated cy/+ rats, however, have fibrotic kidneys [24] and treatment of cy/+ rats with PLX5568 further increased kidney fibrosis in our experiments. Therefore, it is intriguing to speculate that pro-fibrotic processes need to be initiated to some extent prior to application of PLX5568, which are then aggravated. Upregulation of PAI 1 upon treatment with PLX5568 supports the idea of Transforming Growth Factor-beta-mediated fibrosis in cy/+ rats [37] potentially aggravated by PLX5568. Further studies will be needed in other animals to determine whether aggravated fibrosis is limited to this disease model or may also occur in other models. In addition, it will be interesting to see in future studies with other compounds whether the induction of fibrosis is limited to PLX5568 or inhibition of Raf kinases in general. The former may be more likely, as Sorafenib, a
small molecule Raf inhibitor is anti-fibrotic on stellate cells and in case of liver fibrosis [38]. Recently, Sorafenib has been shown to inhibit in vitro cyst growth of human ADPKD cyst epithelial cells [20]. It would be interesting to examine the effects of Sorafenib in PKD animal models in regard to cyst growth but also renal fibrosis.

Interestingly, inhibition of cyst cell proliferation by inhibition of mammalian target of rapamycin (mTOR) reduces cyst sizes in human patients without improvement of kidney function [39], also suggesting that kidney function may not be completely dependent on the size of the cysts but rather a combination of cyst growth and factors within the kidney that promote interstitial fibrosis, the final common pathway in chronic kidney diseases.

In summary, we provide the first data that inhibition of Raf kinases with PLX5628 may help to attenuate cyst growth in PKD but may bear the risk of adverse effects like organ fibrosis balancing the potential benefit of reduced cyst sizes.

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Conflict of interest statement. G.B., J.T. and P.H. are employees of Plexxikon, Berkeley. D.W. received a grant from Plexxikon.

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Protective effects of L-type fatty acid-binding protein (L-FABP) in proximal tubular cells against glomerular injury in anti-GBM antibody-mediated glomerulonephritis

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Abstract

Background. In glomerulonephritis (GN), an overload of free fatty acids (FFA) bound to albumin in urinary protein may induce oxidative stress in the proximal tubules. Human liver-type fatty acid-binding protein (hL-FABP) expressed in human proximal tubules, but not rodents, participates in intracellular FFA metabolism and exerts anti-oxidative effects on the progression of tubulointerstitial damage. We examined whether tubular enhancement of this anti-oxidative action modulates the progression of glomerular damage in immune-mediated GN in hL-FABP chromosomal gene transgenic (Tg) mice.

Methods. Anti-glomerular basement membrane antibody-induced glomerulonephritis (anti-GBM GN) was induced in Tg and wild-type mice (WT). Proteinuria, histopathology, polymorphonuclear (PMN) influx, expression of tubulointerstitial markers for oxidative stress 4-hydroxy-2-Nonenal (HNE) and fibrosis (α-smooth muscle actin), proximal tubular damage (Kim-1), Peroxisome Proliferator-Activated Receptor γ (PPARγ) and inflammatory cytokines [Monocyte Chemotactic Protein-1, tumor necrosis factor-alpha (TNF-α) and Transforming growth factor beta (TGF-β)] were analyzed. The mice were also treated with an angiotensin type II receptor blocker (ARB)

Results. The urinary protein level in Tg mice decreased significantly during the acute phase (~Day 5). Tg mice survived for a significantly longer time than WT mice, with an attenuation of tubulointerstitial damage score and expression of each tubulointerstitial damage marker observed at Day 7. Expression of inflammatory cytokines on Day 7 was higher in WT mice than Tg mice and correlated strongly with PPARγ expression in WT mice, but not in Tg mice. Interestingly, Tg mice showed insufficient PMN influx at 3 and 6 h, with simultaneous elevation of urinary L-FABP and reduction in HNE expression. The two strains of mice showed different types of glomerular damage, with mild mesangial proliferation in Tg mice and severe endothelial swelling with vascular thrombosis in WT mice. The glomerular damage in Tg mice was improved by administration of an ARB.

Conclusions. The present experimental model suggests that tubular enhancement of L-FABP may protect mice with anti-GBM GN from progression of both tubulointerstitial and glomerular injury.

Keywords: L-FABP; oxidative stress; tubulointerstitial damage

Introduction

Recent studies have shown that urinary protein is renotoxic and contribute to the progression of renal injury by causing tubulointerstitial abnormalities [1–5]. In massive proteinuria, there is an overload of free fatty acids (FFAs) bound to albumin in the proximal tubules. This induces production of certain inflammatory factors such as macrophage chemotactic factors, which in turn exacerbates urinary protein-related tubulointerstitial damage [6–9]. Oxidative stress in proximal tubules is induced by FFAs by the process of protein reabsorption. Oxidative stress is considered as one of the major causes of tubulointerstitial injury [10–13]. Following accumulation of FFAs in the proximal tubules,