Delayed Administration of Suramin Attenuates the Progression of Renal Fibrosis in Obstructive Nephropathy

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ABSTRACT

We recently showed that suramin treatment prevents the onset of renal fibrosis in a model of obstructive nephropathy induced by unilateral ureteral obstruction (UUO). In this study, we further assessed the effect of delayed administration of suramin on the progression of tubulointerstitial fibrosis. Mice were given a single dose of suramin at 20 mg/kg starting at day 3 of obstruction, and kidneys were harvested after an additional 7 or 14 days of obstruction. Suramin completely blocked further increase in expression of type I collagen and fibronectin and largely suppressed expression of α -smooth muscle actin (α -SMA) in both treatment groups. UUO injury induced phosphorylation of Smad-3, a key mediator of transforming growth factor- β (TGF- β) signaling, epidermal growth factor receptor, and platelet-derived growth factor receptor after 3 days and further

increased at 10 days after UUO injury. When suramin was administered at 3 days after obstruction, phosphorylation of these molecules was not further increased in the obstructed kidney. Suramin treatment also inhibited activation of signal transducer and activator of transcription 3 and extracellular signal-regulated kinase 1 and 2, two signaling pathways associated with renal fibrogenesis. Furthermore, delayed application of suramin suppressed TGF- β 1-induced expression of α -SMA and fibronectin in cultured renal interstitial fibroblasts. These results indicate that administration of suramin is effective in attenuating the progression of renal fibrosis after injury and suggest the potential clinical application of suramin as an antifibrotic treatment in patients with chronic kidney disease.

Introduction

Chronic kidney disease (CKD) is the result of various insults to the kidney, affecting approximately 10% of the normal population. It is a progressive process marked by interstitial fibrosis (Neilson, 2006; Wynn, 2008). Development and progression of renal fibrosis is the end result of activation of a wide variety of growth factor and cytokine receptors such as transforming growth factor- β (TGF- β) receptor, platelet-derived growth factor receptor (PDGFR) (Bonner, 2004), and epidermal growth factor receptor (EGFR) (Terzi et al., 2000; Lautrette et al., 2005). Activation of TGF- β receptors leads to activation of several intracellular signaling pathways, including Smad-3 (Wang et al., 2005), signal transducer and

activator of transcription 3 (STAT3) (Qin et al., 2009), and extracellular signal-regulated kinase 1 and 2 (ERK1/2) (Song et al., 2007; Suzuki et al., 2007). These signaling pathways induce activation of renal fibroblasts, leading to excessive accumulation of extracellular matrix (ECM) components including type I collagen and fibronectin in the kidney. PDGFR and EGFR play an important role in activation of STAT3 and ERK1/2 signaling pathways (Vignais et al., 1996; Lo et al., 2007).

CKD may progress to the need for renal replacement therapy in the end stage of renal disease (ESRD). Given the high prevalence of CKD and cost of replacement therapies for ESRD, any treatment that halts or slows the progression of renal fibrosis has the potential to provide an immense medical, social, and economic benefit. At present, angiotensin-converting enzyme inhibitors and angiotensin II receptor type 1 blockers are clinically used to combat renal fibrosis (Locatelli et al., 2009; Morrow et al., 2010). However, these drugs are not able to completely stop the progression of renal fibrosis; in some conditions, such as aristolochic acid-induced

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ABBREVIATIONS: CKD, chronic kidney disease; TGF- β , transforming growth factor- β ; PDGFR, platelet-derived growth factor receptor; EGFR, epidermal growth factor receptor; STAT3, signal transducer and activator of transcription 3; ERK1/2, extracellular regulated kinase 1 and 2; ECM, extracellular matrix; ESRD, end stage of renal disease; UUO, unilateral ureteral obstruction; p, phospho; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; α -SMA, α -smooth muscle actin; NRK-49F, normal rat kidney fibroblast; FBS, fetal bovine serum.

renal fibrosis in rats, they are not effective at all (Debelle et al., 2004; Boor et al., 2007). Because renal fibrogenesis is a complex process that is involved in the activation of multiple cellular and molecular mediators, the incomplete antifibrotic effect of angiotensin-converting enzyme inhibitors and angiotensin II receptor type 1 blockers may be due to their limited targets. As such, an agent that inhibits multiple profibrotic signaling pathways might offer improved therapeutic benefit in fibrotic kidney disease.

It has been reported that suramin, a polysulfonated naphthylurea, has a broad inhibitory effect on the action of growth factor/cytokine receptors (Coffey et al., 1987; Stein et al., 1989; Kloen et al., 1994; Fujiuchi et al., 1997; Abdiu et al., 1999) by competitive binding to cytokine/growth factor receptors (Stein, 1993; Zunkeller and Schofield, 1995). Studies on animal models have shown that administration of suramin

attenuates muscle and liver fibrosis (Chan et al., 2003; Li et al., 2009). Furthermore, suramin can prevent coronary restenosis after percutaneous transluminal coronary angioplasty (Urasawa et al., 2001). In a recent study, we also assessed the effect of suramin on the development of renal fibrosis in animal models of obstructive nephropathy and remnant kidney diseases and demonstrated that it was effective in inhibiting renal fibrosis when given before initiation of renal fibrosis (Liu et al., 2011).

Because patients with CKD usually have a certain degree of renal fibrosis, it will be useful if suramin is capable of halting or slowing the progression of renal fibrosis in established tubulointerstitial fibrosis. To address this issue, we designed experiments to assess the therapeutic effect of delayed administration of suramin on the progression of tubulointerstitial fibrosis in the mouse model of kidney fibrosis induced by UUO.

Materials and Methods

Antibodies and Chemicals. Antibodies to p-STAT3, STAT3, p-Smad-3, Smad-3, p-ERK1/2, ERK1/2, p-EGFR, p-PDGFR β , and PDGFR β were purchased from Cell Signaling Technology (Danvers, MA). Antibodies to fibronectin, collagen 1(A2), GAPDH, and EGFR were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Suramin, antibodies to α -smooth muscle actin (α -SMA) and α -tubulin, and all other chemicals were from Sigma-Aldrich (St. Louis, MO).

Fig. 1. Experimental design. Experiments were designed to evaluate the effect of suramin given at 3 days of obstruction on the progression of renal fibrosis.

Fig. 2. Time course of UUO-induced expression of type I collagen, fibronectin, and α -SMA. The left ureter was ligated. At days 1, 3, 7, and 14, the kidneys were taken for analyzing the expression of type I collagen, fibronectin, α -SMA, and GAPDH. Representative immunoblots from three or more experiments are shown (A). Expression levels of fibronectin (B), type I collagen (C), and α -SMA (D) were quantified by densitometry and normalized with GAPDH. Data are means $\pm$ S.E.M. ($n = 6$). Means with different superscript letters are significantly different from one another ($P < 0.05$).

Fig. 3. Effect of delayed suramin administration on UUO-induced deposition of ECM and expression of type I collagen and fibronectin. Mice received surgery and suramin treatment as described in the animal protocol described under *Materials and Methods*. A, photomicrographs illustrating Masson trichrome staining of kidney tissue after various treatments. B, the graph shows the percentage of Masson trichrome-positive tubulointerstitial area (blue) relative to the whole area from 10 random cortical fields (200 $\times$) (means $\pm$ S.E.M.). C, kidney tissue lysates were subjected to immunoblot analysis with specific antibodies against type I collagen, fibronectin, or GAPDH. Expression levels of fibronectin (D) and type I collagen (E) were quantified by densitometry and normalized with GAPDH. Data are means $\pm$ S.E.M. ($n = 6$). Means with different superscript letters are significantly different from one another ($P < 0.05$).

Fig. 4. Effect of delayed suramin administration on UUO-induced expression of α -SMA. Mice received surgery and suramin treatment as described in the legend to Fig. 1. A, kidney tissue lysates were subjected to immunoblot analysis with specific antibodies against α -SMA or GAPDH. B, expression levels of α -SMA was quantified by densitometry and normalized with GAPDH. Data are means $\pm$ S.E.M. ($n = 6$). Means with different superscript letters are significantly different from one another ($P < 0.05$).

Animals and the UUO Model. The UUO model was established in male C57 back mice that weighed 20 to 25 g (The Jackson Laboratory, Bar Harbor, ME) as described in our previous study (Pang et al., 2009). The animal protocol was approved by the Animal Care Committee of Rhode Island Hospital (Providence, RI). In brief, the abdominal cavity was exposed via a midline incision, and the left ureter was isolated and ligated. Sham-operated animals received the same surgical procedures but without ureter ligation. To establish the time course of renal fibrogenesis in this model, four groups of mice ($n = 5$) were killed at days 1, 3, 7, and 14, respectively, after left ureter ligation. To examine the effect of suramin on the progression of renal fibrosis, suramin was administered on day 3 after obstruction (Fig. 1): groups I and II were sham-operated or UUO mice killed at 3 days after surgery; groups III and IV were mice that underwent UUO injury and were given either vehicle or suramin from day 3 to 10 (1 week); and groups V and VI were mice that underwent UUO injury and given either vehicle or suramin from day 3 to 17 (2 weeks). A single dose of suramin (20 mg/kg) was administered by intraperitoneal injection. Control mice were injected with the equal volume of vehicle (0.9% saline). At the end of the experiments, mice were killed, and the kidneys were removed. One part of the kidneys was fixed in 10% phosphate-buffered formalin for histological studies. Another part was snap-frozen in liquid nitrogen and stored at -80°C for protein extractions.

Cell Culture and Treatments. Normal rat kidney fibroblast (NRK-49F) cells were cultured in Dulbecco's modified Eagle's medium (Sigma-Aldrich) containing 5% fetal bovine serum (FBS), and 0.5% penicillin and streptomycin in an atmosphere of 5% CO_2 and 95% air at 37°C . To determine the effects of delayed suramin treatment on fibroblast activation, NRK-49F cells were starved for 24 h by incubation with Dulbecco's modified Eagle's medium containing

0.5% FBS followed by stimulation with TGF- β 1 (1 ng/ml) for 24 or 48 h and then incubated with 100 μ M suramin for an additional 24 or 48 h.

Immunoblot Analysis. Immunoblot analysis of NRK-49F cells and tissue samples were conducted as described previously (Pang et al., 2009). The densitometry analysis of immunoblot results was conducted by using NIH Image software (National Institutes of Health, Bethesda, MD).

Immunohistochemical Staining. Immunohistochemical staining was performed according to the procedure described in our previous studies (Pang et al., 2009). For assessment of renal fibrosis, Masson trichrome staining was performed according to the protocol provided by the manufacturer (Sigma-Aldrich). The collagen tissue area (blue color) was quantitatively measured using Image-Pro Plus software (Media Cybernetics, Inc., Silver Spring, MD) by drawing a line around the perimeter of positive staining area, and the average ratio to each microscopic field (400 $\times$) was calculated and graphed.

Statistical Analysis. All the experiments were conducted at least three times. Data depicted in graphs represent the means $\pm$ S.E.M. for each group. Intergroup comparisons were made using one-way analysis of variance. Multiple means were compared using Tukey's

test. The differences between two groups were determined by Student's *t* test. A statistically significant difference between mean values was marked in each graph. *P* < 0.05 is considered significant.

Results

UUO Injury Induces Expression of Type I Collagen, Fibronectin, and α -SMA in a Time-Dependent Manner.

Renal fibrosis is characterized by overproduction of ECM proteins that include type I collagen and fibronectin and by activation of renal interstitial fibroblasts (Liu, 2006). As the first step toward understanding the effect of delayed administration of suramin on renal fibrosis, we examined expression of type I collagen and fibronectin over time after obstructive injury. As shown in Fig. 2, basal levels of fibronectin were not detected in the sham-operated animals. After ureteral obstruction, fibronectin was detectable on day 3 but was significantly increased on day 7 and further elevated on day 14. In contrast, an abundance of type I collagen was ex-

Fig. 5. The long-term effect of delayed suramin administration on UUO-induced expression of type I collagen, fibronectin, and α -SMA. Mice received surgery and suramin treatment as described in Fig. 1. Kidney tissue lysates were subjected to immunoblot analysis with specific antibodies against type I collagen, fibronectin, α -SMA, or α -tubulin (A). Expression levels of fibronectin (B), type I collagen (C), and α -SMA (D) were quantified by densitometry and normalized with α -tubulin. Data are means $\pm$ S.E.M. (*n* = 6). Means with different superscript letters are significantly different from one another (*P* < 0.05).

pressed in the sham-operated kidneys, and its expression was increased on day 3 and further elevated on days 7 and 14 after obstruction. To confirm the above observation, we also examined interstitial ECM deposition by Masson trichrome staining. An increase in Masson trichrome-positive staining was observed within the tubulointerstitial area on day 3 after UVO injury, and these areas were expanded over time from 7 to 14 days (Fig. 3A and data not shown).

Myofibroblasts are the principal cells known to be responsible for accumulation and deposition of the interstitial matrix and characterized by expression of α -SMA (Neilson, 2006; Wynn, 2008). We also examined its kinetic expression after UVO injury. As shown in Fig. 2, A and D, α -SMA was barely detected in the sham-operated kidney. Obstructive injury induced an increase in expression of α -SMA on day 3 that was further increased on days 7 and 14 after obstruction. Quantitative determination of Western blot analysis showed that induction of α -SMA expression level was increased up to 5-fold at day 3 after obstructive injury and approximately 30- and 48-fold increase of α -SMA expression was observed on days 7 and 14, respectively.

Effect of Delayed Administration of Suramin (Starting 3 Days after Obstruction) on the Progression of Renal Fibrosis. To compare the therapeutic effect of suramin on the progression of renal fibrosis, we decided to start suramin treatment at day 3 after UVO injury. After treatment with suramin for 7 and 14 days, mice were sacrificed, and kidneys were removed to assess the deposition of ECM by Masson trichrome staining and expression of type I collagen and fibronectin by immunoblot analysis.

An increase in Masson trichrome-positive areas was observed within the tubulointerstitium 3 days after the onset of obstruction and further increased at 10 days of obstruction. The obstructed kidneys of mice treated with suramin starting at day 3 after UVO showed a decrease in Masson trichrome-positive areas. Semiquantitative analysis revealed a 2.2-fold increase in Masson trichrome-positive areas in the obstructed kidney on day 10 compared with that on day 3. In the obstructed kidney treated with suramin, Masson trichrome-positive areas were reduced on day 10 to a level similar to that in the kidney at 3 days of obstruction (Fig. 3, A and B). Similar results were also obtained in the kidney treated for 14 days starting from day 3 and ending at day 17 after onset of obstruction (data not shown). Consistent with these results, immunoblot analysis showed that administration of suramin at day 3 of obstruction also reduced collagen type I and fibronectin expression to the starting levels in both 7- or 14-day treatment groups (Figs. 3 and 5). Suramin treatment in those two groups also reduced α -SMA expression (Figs. 4 and 5). These data suggest that suramin is a potent inhibitor that blocks the progression of renal fibrosis after injury.

Effect of Delayed Administration of Suramin on UVO-Induced Phosphorylation of Smad-3. To examine the mechanism involved in the antifibrotic effect of suramin when administered after UVO induction, we first analyzed the effect of suramin on the activation of Smad-3, a key mediator of TGF- β signaling. Figure 6 shows that after UVO injury, the phosphorylation level of Smad-3 was increased at day 3 and further increased at day 10. Treatment with suramin at 3 days after UVO injury largely blocked Smad-3 phosphorylation. These data suggest that suramin is able to

Fig. 6. Effect of delayed suramin administration on UVO-induced phosphorylation of Smad-3. Mice received surgery and suramin treatment as described in Fig. 1. A, kidney tissue lysates were subjected to immunoblot analysis with specific antibodies against p-Smad-3 or Smad-3. B, p-Smad-3 was quantified by densitometry and normalized with and Smad-3. Data are means $\pm$ S.E.M. ($n = 6$). Means with different superscript letters are significantly different from one another ($P < 0.05$).

inhibit the activation of TGF- β signaling when given after obstruction.

Effect of Delayed Administration of Suramin on Phosphorylation of EGFR and PDGFR in the Obstructed Kidney. Because activation of EGFR and PDGFR also contributes to renal fibrosis (Terzi et al., 2000; Bonner, 2004; Lautrette et al., 2005), we further examined the effect of suramin on the phosphorylation of EGFR and PDGFR. At day 10 after surgery, the obstructed kidney showed a significant increase in the phosphorylation of EGFR and PDGFR compared with that in the obstructed kidney at day 3. Administration of suramin at day 3 of obstruction resulted in a significant reduction of EGFR and PDGFR phosphorylation (Fig. 7, A, B, and D). Although UVO injury also induced an increase in expression of total EGFR and PDGFR, these expression levels were not affected by suramin (Fig. 7, A, C, and E). These data suggest that delayed administration of suramin blocks UVO injury-induced phosphorylation of both EGFR and PDGFR in the obstructive kidney.

Effect of Delayed Administration of Suramin on Phosphorylation of STAT3 and ERK1/2 in the Obstructive Kidney. Activation of STAT3 and ERK1/2 is associated with renal fibrosis and is downstream of multiple membrane receptors including EGFR and PDGFR (Song et al., 2007; Suzuki et al., 2007; Qin et al., 2009). We thus analyzed their phosphorylation state by immunoblot analysis in kidney tissue. As shown in Fig. 8, UVO injury induced phosphorylation of both STAT3 and ERK1/2 after 3 days that further increased at 10 days after UVO injury. When suramin was administered at 3 days after obstruction, their phosphorylation was not further increased (Fig. 8, A, B, and D). Although UVO injury also increased total protein levels of these two molecules, suramin treatment did not alter their expression levels. These data indicate that suramin is able to suppress the activation of these cellular signaling pathways after UVO injury.

Fig. 7. Effect of delayed suramin administration on UVO-induced phosphorylation of EGFR and PDGFR β . Mice received surgery and suramin treatment as described in Fig. 1. A, kidney tissue lysates were subjected to immunoblot analysis with specific antibodies against p-PDGFR β , PDGFR β , p-EGFR, EGFR, or GAPDH. Expression levels of p-PDGFR β (B) and p-EGFR (D) were quantified by densitometry and normalized with PDGFR β or EGFR. Expression levels of PDGFR β (C) and EGFR (E) were quantified by densitometry and normalized with GAPDH. Data are means $\pm$ S.E.M. ($n = 6$). Means with different superscript letters are significantly different from one another ($P < 0.05$).

Delayed Treatment with Suramin Inhibits Expression of Fibronectin and α -SMA in Cultured Renal Interstitial Fibroblasts. To demonstrate the inhibitory effect of suramin on activation of renal interstitial fibroblasts in vitro, we designed a treatment scheme to mimic in vivo situations as described in this study (Fig. 9A). NRK-49F cells were pretreated with TGF- β 1 (1 ng/ml) for 24 and 48 h and then were exposed to suramin (100 μ M) for an additional 24 and 48 h in the continuous presence of TGF- β 1 (1 ng/ml). At 48 h after pretreatment with TGF- β 1, expression of α -SMA and fibronectin was induced, which is similar to the in vivo situation. TGF- β 1 further increased expression of these two proteins in the absence of suramin; however, delayed treatment with suramin suppressed further increases in these two proteins. These data suggest that delayed administration of suramin is effective in suppressing activation of renal interstitial fibroblasts induced by TGF- β 1.

Discussion

In a recent study, we found that suramin is effective in preventing the onset of renal fibrosis in a model of obstructive nephropathy when given immediately after surgical li-

gation of the ureter (Liu et al., 2011). In the present study, we further demonstrate that delayed administration of a single dose of suramin after UVO injury inhibits further increases in renal fibrosis as indicated by reduced deposition of ECM components and expression of type I collagen and fibronectin. Collectively, these results indicate that suramin treatment not only blocks development of renal fibrosis when given at the early time but also attenuates the progression of renal fibrosis after a significant degree of tubulointerstitial fibrosis has occurred.

The mechanism by which delayed administration of suramin attenuates renal fibrosis is not fully understood. Because α -SMA-positive myofibroblasts are the principal effector cells responsible for ECM overproduction in the fibrotic kidney (Wynn, 2008), we examined the effect of suramin on their activation in vivo and in vitro. Our data show that suramin administrated at day 3 after UVO injury largely reduced expression of α -SMA in vivo and suramin treatment also inhibited TGF- β 1-induced expression of α -SMA in cultured NRK-49F cells, suggesting that blockade of renal fibroblast activation may be a critical step for suramin to inhibit the deposition of ECM proteins and the progression of renal

Fig. 8. Effect of delayed suramin administration on UUO-induced phosphorylation of STAT3 and ERK1/2. Mice received surgery and suramin treatment as described in Fig. 1. A, kidney tissue lysates were subjected to immunoblot analysis with specific antibodies against p-STAT3, STAT3, p-ERK1/2, ERK1/2, or GAPDH. Expression levels of p-STAT3 (B) and p-ERK1/2 (D) were quantified by densitometry and normalized with STAT3 and ERK1/2. Expression levels of STAT3 (C) and ERK1/2 (E) were quantified by densitometry and normalized with GAPDH. Data are means $\pm$ S.E.M. ($n = 6$). Means with different superscript letters are significantly different from one another ($P < 0.05$).

Fig. 9. Delayed administration of suramin inhibits TGF- β 1-induced α -SMA and fibronectin expression in renal interstitial fibroblasts. A, diagram depicts treatment scheme with suramin, which were designed to mimic suramin treatment scheme illustrated in Fig. 1. B, immunoblot analysis shows that the levels of α -SMA and fibronectin protein in NRK-49F cells after various treatments as indicated. Expression levels of fibronectin (C) and α -SMA (D) were quantified by densitometry and normalized with α -tubulin. Data are means $\pm$ S.E.M. ($n = 6$). Means with different superscript letters are significantly different from one another ($P < 0.05$).

fibrosis. However, because our studies showed that suramin inhibits the expression of ECM proteins to a greater degree than inhibition of α -SMA expression, suramin may also inhibit the production and deposition of ECM proteins through a mechanism independent of renal fibroblast activation. Fur-

ther studies are needed to examine the effect of suramin on the expression of ECM proteins in different renal cell types.

Progression of renal fibrosis is a complicated process with a variety of cellular and molecular mediators interacting in concert (Neilson, 2006; Wynn, 2008). Previous studies have

shown that activation of EGFR and PDGFR as well as TGF- β signaling plays a prominent role in this process (Terzi et al., 2000; Bonner, 2004; Lautrette et al., 2005; Liu, 2006). As such, we examined the effect of delayed suramin treatment on activation of these signaling pathways. Our data clearly show that starting suramin treatment at day 3 after injury inhibited the phosphorylation of Smad-3 to the level at the initial time of injury. In addition, suramin equally inhibited phosphorylation of EGFR and PDGFR in UUO-injured animals. Therefore, the inhibition of those receptors may account, at least in part, for the antifibrotic effect of suramin observed after UUO injury. Nevertheless, suramin is an inhibitor with broad specificity and also inhibits other membrane receptors such as purinergic receptor P2X₇ and sphingosine-1-phosphate receptors (Goncalves et al., 2006; Li et al., 2009). Because these two receptors are also associated with development and progression of tissue fibrosis, it will be interesting to further investigate whether blockade of these receptors by suramin contributes to its antifibrotic effects.

It has been reported that activation of ERK1/2 and STAT3 pathways is involved in the development/progression of kidney fibrosis. Previous studies have shown that activated ERK1/2 is prominent in tubules and interstitial cells in areas of tubulointerstitial damage in a variety of human glomerulopathies (Masaki et al., 2004) and are required for activation of renal fibroblasts and expression of ECM proteins (Sekine et al., 2003; Suzuki et al., 2004). Our recent work also demonstrated that STAT3 was persistently activated after obstructive injury and is involved in the activation of renal interstitial fibroblasts, accumulation of inflammatory cells and expression of TGF- β and TGF- β RII, and increase in some proinflammatory mediators such as tumor necrosis factor- α , interleukin-1 β , and intercellular adhesion molecule-1 (Pang et al., 2010). Because both STAT3 and ERK1/2 pathways are activated in response to a variety of growth factor/cytokine receptors (Kim and Choi, 2010; Ivashkiv and Hu, 2004), inhibition of these signaling pathways by suramin may be the event due to inactivation of some membrane receptors such as TGF- β , epidermal growth factor, and platelet-derived growth factor receptors. These data, together with suramin-mediated blockade of Smad-3 activation suggest that suramin administration is able to simultaneously shut down multiple signaling pathways that contribute to renal fibrogenesis and provides evidence for the marked effect of suramin in abrogating the progression of renal fibrosis in this model.

The incidence and prevalence of CKD is increasing worldwide. Unchecked progression of CKD invariably leads to ESRD and the requirement for renal replacement therapy (dialysis or transplantation). The primary aims of treatment in patients with CKD are both to prevent or, at least slow, progression of CKD. However, available therapies are limited and include inhibitors of renin/angiotensin system (Locatelli et al., 2009; Morrow et al., 2010). However, these drugs are not able to completely block the progression. Here, we have shown that suramin is able to halt progressive renal fibrosis in a mouse model when given early after UUO injury. Our recent studies also show that suramin inhibited the progression of renal fibrosis and prevented renal function impairment in a rat model of remnant kidney disease (Liu et al., 2011). Therefore, suramin might be an interesting drug candidate for treating patients with CKD.

In summary, our data demonstrate that suramin is effective in blocking subsequent renal fibrosis after the emergence of established fibrosis. The antifibrotic effect of suramin is associated with inhibition of several cellular signaling pathways that contribute to renal fibrosis. Because ESRD is preceded, in nearly all cases, by the progressive development of renal fibrosis, delayed administration of suramin may have therapeutic potential by halting or at least slowing the development of fibrosis.

Authorship Contributions

Participated in research design: Liu, Yan, and Zhuang.

Conducted experiments: Liu, Tolbert, and Ponnusamy.

Contributed new reagents or analytic tools: Liu and Zhuang.

Performed data analysis: Liu and Ponnusamy.

Wrote or contributed to the writing of the manuscript: Liu and Zhuang.

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