Chapter 4

Novel Therapeutic Targets for the Treatment of Tubulointerstitial Fibrosis

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Current Signal Transduction Therapy (accepted)
Abstract

Approximately 80% of the kidney is composed of tubular cells which secret and reabsorb substances to and from the urine. Activated tubular cells play a pivotal role in the etiology of renal fibrosis. During renal injury, these activated tubular cells participate in the initiation of fibrogenic processes which eventually may lead to tubulointerstitial fibrosis and end stage renal disease (ESRD). Current therapies such as angiotensin converting enzyme inhibitors, angiotensin II receptor type-1 antagonists and statins do not suffice for the treatment of renal fibrosis. However, in recent years, better understanding of disease mechanisms led to the development of new drug entities that intervene in the signal transduction pathways involved in the disease pathogenesis. This review discusses possible new drugs directed to intracellular signal transduction pathways such as mitogen–activated protein kinases (p38, ERK and JNK), growth factors receptor tyrosine kinases (TGF-β and PDGF), Rho kinase, and nuclear transcription factors that are activated during disease. In addition to kinase inhibitors, novel approaches such as renal selective drug targeting, recombinant protein antifibrotic agents and gene silencing concepts are discussed.
Introduction

There is a tremendous increase in number of patients world-wide with end stage renal diseases (ESRD). Recent data show that the incidence of ESRD in European countries has increased to about 135 cases per million per year, albeit below the rates in the USA (336 per million per year) (1). In most of the developed countries, there is an expected increase in the annual incidence rate of the diseases of 5 to 8%. In relation to this, an increase in the annual expenditure is expected from US$9 billion (1995) to US$28 billion by 2010 (costs in the USA) (1,2). ESRD are the consequences of diabetic nephropathy, chronic glomerulonephritis, tubulointerstitial fibrosis and hypertensive nephrosclerosis. The largely increased knowledge about the mechanisms of interstitial fibrosis and renal function loss has elicited novel approaches to treat these renal diseases (3). In general, tubulointerstitial fibrosis is considered as a common endpoint of several pathological events in the kidney. The tubulointerstitial injury may be initiated by primary renal diseases or may be instigated by secondary processes of progressive glomerular diseases (4). Present research topics are focused on the understanding of the molecular mechanisms involved in the initiation and progression of these diseases. In this frame work, many new potential drug candidates, particularly those affecting signaling cascades have been recently tested in preclinical studies. Such compounds have not advanced into the clinic yet. However, they are seen as potentially important and complimentary to antihypertensive approaches such as angiotensin converting enzyme (ACE) inhibitors and angiotensin II type-1 receptor blockers (ARBs) that are commonly advocated for the treatment of renal fibrosis (5,6). In this review, we will briefly discuss the pathophysiological pathways involved in the initiation of tubulointerstitial fibrosis and we will particularly focus on the various novel therapeutic strategies, including signal transduction interventions and advanced technologies involving macromolecular therapeutics in the field of drug delivery as well as gene and antisense concepts.

Pathophysiologic mechanisms leading to tubulointerstitial fibrosis

Excessive urinary excretion of proteins (proteinuria) is a hallmark of renal injury. After an initial renal insult, either immunologic or non-immunologic, distinct molecules such as albumin, transferrin, immunoglobulins, complement factors, growth factors, angiotensin-II (Ang II), cytokines and high glucose filter through the glomerulus and subsequently interact with renal tubular cells (7), as described in Fig. 1. These molecules activate tubular cells via diverse signaling cascades. In addition, hypoxia, oxidative stress, and many other factors which are induced during pathological conditions stimulate pro-inflammatory and profibrotic signaling pathways in tubular cells. Table 1 summarizes the effect of these activating factors on renal tubular cells in vitro and related pathways involved in the cellular response.
### Table 1. Activation of renal tubular cells against various stimuli in vitro.

<table>
<thead>
<tr>
<th>Stimuli</th>
<th>Signal/pathway</th>
<th>Response</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>High glucose</td>
<td>Increase in transglutaminase activity</td>
<td>Increase in ECM</td>
<td>(195)</td>
</tr>
<tr>
<td></td>
<td>Activation of ERK and p38 MAPK</td>
<td>Induces of TGF-β1 expression, hypertrophy</td>
<td>(196,197)</td>
</tr>
<tr>
<td></td>
<td>Activation of p38 MAPK</td>
<td>Enhances angiotensinogen gene expression</td>
<td>(198)</td>
</tr>
<tr>
<td></td>
<td>Activation of smad2/3</td>
<td>TGF-β1 production (early)</td>
<td>(199)</td>
</tr>
<tr>
<td></td>
<td>Polyl pathway dependent</td>
<td>Increase collagen-1 exp. (late)</td>
<td></td>
</tr>
<tr>
<td>Hemin</td>
<td>Hemeoxygenase-1 dependent activation of p21</td>
<td>Fibronectin accumulation</td>
<td>(200)</td>
</tr>
<tr>
<td>Low O2 plus high CO2</td>
<td></td>
<td>Apoptosis</td>
<td>(201)</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>Phosphatidylinositol 3-kinase (PI3K) dependent</td>
<td>Production of HIF-1α and VEGF</td>
<td>(203)</td>
</tr>
<tr>
<td></td>
<td>Via activation of NF-κB</td>
<td>Induces synthesis of ICAM-1</td>
<td>(204)</td>
</tr>
<tr>
<td>Thrombin</td>
<td>Partly TGF-β1 dependent</td>
<td>Fibronectin production</td>
<td>(205)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MCP-1 and TGF-β1 secretion</td>
<td>(206)</td>
</tr>
<tr>
<td>Oleate-complexed rHSA</td>
<td>Protein kinase C (PKC) activation</td>
<td>Fibronectin secretion</td>
<td>(207)</td>
</tr>
<tr>
<td>Albumin</td>
<td>PKC and tyrosine kinase dependent</td>
<td>Increase in NF-kB and TNF-α specific mRNA expression</td>
<td>(208)</td>
</tr>
<tr>
<td></td>
<td>Via p44/42 (ERK) MAPK pathway activation</td>
<td>Causes cell proliferation</td>
<td>(209)</td>
</tr>
<tr>
<td></td>
<td>NF-κB activation</td>
<td>Increase in H2O2 and ROS production</td>
<td>(8,9)</td>
</tr>
<tr>
<td></td>
<td>PKC and NAD(P)H oxidase dependent</td>
<td>Enhances RANTES and MCP-1 expression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Via p44/42 (ERK) MAPK pathway activation</td>
<td>Increase in TGF-β1 and MCP-1 expression</td>
<td>(210,211)</td>
</tr>
<tr>
<td>Insulin</td>
<td>Post-transcriptional pathway</td>
<td>Increase in the production of TGF-β1 and expression of type-IV collagen</td>
<td>(212)</td>
</tr>
<tr>
<td>Transferrin</td>
<td></td>
<td>Increase in expression of complement C3</td>
<td>(213)</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>Activation of smad2/3</td>
<td>Transdifferentiation of the cells into myofibroblasts</td>
<td>(214-216)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Induces α-smooth muscle actin, collagens expression and loss of e-cadherin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mediated via PKC</td>
<td>Release preformed bFGF</td>
<td>(217)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Induces VEGF expression</td>
<td>(218)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Activation of p38 and ERK MAPK</td>
<td>IL-6 production</td>
<td>(219)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increase in IL-8 expression</td>
<td>(220)</td>
</tr>
</tbody>
</table>
Filtered albumin acts on tubular cells through unknown mechanisms and, among other effects, generates reactive oxygen species which in turn activate the transcription factor nuclear factor-κB (NF-κB) within tubular cells. Activation of this pathway leads to the production of various chemokines and cytokines such as monocyte chemoattractant protein-1 (MCP-1), regulated upon activation normal T-cell expressed and secreted (RANTES) and transforming growth factor-β (TGF-β) (8-10). Chemoattractants such as MCP-1 and RANTES in turn facilitate the infiltration of monocytes and neutrophils from the systemic circulation into tubulointerstitial space that surround the activated resident cells. These infiltrated macrophages augment the fibrogenic response of interstitial fibroblasts by generating various profibrotic factors, including TGF-β, tumor necrotic factor-α (TNF-α), and endothelin-1, which are instrumental in the synthesis of extracellular matrix (ECM). Macrophages are also involved in the production of inhibitors of matrix degrading proteases such as tissue inhibitor of metalloproteinase-1 (TIMP-1) and plasminogen activator inhibitor-1 (PAI-1) (11,12). Other factors, for instance TGF-β1, either secreted from resident tubular cells or filtered through glomeruli, bind to TGF-β type-II receptors present on tubular epithelial cells and interstitial fibroblasts. This also initiates several fibrotic events such as transformation of fibroblasts into myofibroblasts (3), production of inhibitors of matrix degrading enzymes, transdifferentiation of tubular cells into fibroblasts (epithelial mesenchymal transformation), and further chemotaxis of inflammatory cells. Interstitial myofibroblasts produce ECM proteins including collagens and fibronectin as

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## Novel therapeutic targets for the treatment of renal fibrosis

<table>
<thead>
<tr>
<th>Stimuli</th>
<th>Signal/pathway</th>
<th>Response</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin-II</td>
<td>Activation of ERK, p38 and P27kip1 Partly by TGF-β1 activation</td>
<td>Cellular hypertrophy Induces collagen α3(IV) expression</td>
<td>(221,222) (223,224)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>TGF-β1 dependent</td>
<td>Increase in IL-8 expression Enhances α-smooth muscle actin and fibronectin expression</td>
<td>(220) (225)</td>
</tr>
<tr>
<td>LPS</td>
<td>NF-kB dependent</td>
<td>Increase in IL-8 expression Induce MCP-1 expression</td>
<td>(220) (226)</td>
</tr>
<tr>
<td>Advanced glycation end-product modified albumin</td>
<td></td>
<td>Inhibit NO and NO synthase enzyme Stimulates TGF-β expression by overgenerating intracellular ROS</td>
<td>(227) (228)</td>
</tr>
<tr>
<td>CD40 ligands</td>
<td></td>
<td>Increase complement C3 expression</td>
<td>(229)</td>
</tr>
<tr>
<td>CTGF</td>
<td></td>
<td>Increase in TGF-β1 and α-smooth muscle actin expression while decrease in collagen-IV</td>
<td>(230)</td>
</tr>
<tr>
<td>HIV-1 gp120 1, 25-dihydroxyvitamin D3</td>
<td>Phosphorylation of p38 MAPK</td>
<td>apoptosis</td>
<td>(231)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stimulate TGF-β1 synthesis</td>
<td>(232)</td>
</tr>
</tbody>
</table>
essential components of the renal scar tissue. The production of the ECM within the tissue should be seen as a dynamic and tightly regulated process. For instance, parallel to the increased production of ECM components, matrix metalloproteinases (MMPs) are activated from their latent forms that degrade ECM proteins. Plasmin which is produced from its precursor plasminogen also degrades ECM proteins and also facilitates the activation of MMPs. In turn, these matrix degrading MMPs and plasmin are inhibited by TIMP-1 and PAI-1 which are produced by activated macrophages (12). So, activated tubulointerstitial cells stimulate a matrix producing system, a matrix degrading system and inhibitor pathways that control both systems, at the same time. Eventually, it is the imbalance between the producing mechanism and the degradation of ECM that leads to progressive tubulointerstitial fibrosis. Therapeutic approaches may aim at preventing the activation of tubulointerstitial cells by preventing proteinuria, hypertension or tissue damage, or may aim at an intervention of the subsequent events. As outlined above, this is a very complex enterprise.

**Figure 1.** Mechanisms of tubulointerstitial fibrosis. Proteinuria, cytokines or hypoxia generated during renal injury activate proximal tubular cells and in turn, tubular cells produce growth factors, chemokines and adhesion molecules. Chemoattractants such as MCP-1 and RANTES attract monocytes from systemic circulation. These infiltrated macrophages are activated by cytokines and produce subsequently several profibrotic factors which further stimulate fibroblast cells. TGF-β produced by tubular cells or macrophages activated different cell types, leading to fibrogenesis. TGF-β–activated renal epithelial tubular cells undergo epithelial mesenchymal transdifferentiation (EMT) and transform into myofibroblasts. Myofibroblasts produce extracellular matrix (ECM) proteins including fibronectin and collagens in the interstitial space which eventually cause tubulointerstitial fibrosis.
Current Therapies

Angiotensin-II and its blockers

Ang II is a well known vasoconstrictor peptide and also considered as a renal growth factor which participates in many intracellular signaling mechanisms that regulate cell growth and synthesis or degradation of ECM (13). The role of Ang II in the pathogenesis of renal fibrosis has been well established as suggested by the following studies. In proteinuric rats, an increase in ACE activity and local Ang II generation primarily in proximal tubular cells was associated with the induction of tubulointerstitial lesions (14). In addition, infusion of Ang II in rats resulted in tubulointerstitial injury, with tubular atrophy and dilation, cast formation, infiltration of monocytes, and mild interstitial fibrosis with increased type IV collagen deposition (15). Stimulation of Angiotensin II type-1 receptors led to the activation of several intracellular signaling cascades such as protein kinase C, tyrosine kinases, mitogen-activated protein kinases (p38, ERK and JNK) and their downstream nuclear transcription factors, activator protein-1 and NF-κB (Fig. 2) (16). Ang II has also been reported to directly induce TGF-β production in renal tubular cells and fibroblasts. Moreover, blockade of Ang II by administering ACE inhibitor or ARBs reduced TGF-β expression and ameliorated tubulointerstitial fibrosis in different experimental animal models such as unilateral ureteral obstruction (UUO) model, Heymann Nephritis model, cyclosporin nephropathy, etc. (17).

ACE inhibitors and ARBs are generally prescribed as antihypertensive agents that can interfere with Ang II–induced vasoconstriction. Recent clinical trials data demonstrated that both classes of compounds reduced serum creatinine levels, blood pressure and proteinuria in patients with hypertension and primary renal disease (proteinuria >1.5 g/24h) (18). In these studies, ARBs provided a superior renoprotection compared with ACE inhibitors and the combination of ARBs and ACE inhibitors resulted even in additive ameliorative effects. Other studies also reported similar benefits of high-dose combination therapies such as valsartan with benazepril, losartan with lisinopril, and irbesartan with enalapril or fosinopril, in patients with diabetic and non-diabetic renal diseases (19). Unfortunately, these therapies only retard the progression of chronic renal diseases and do not prevent or reverse the renal function loss, indicating the need for additional therapeutic approaches.

Statins

Statins are the competitive inhibitors of 3-hydroxy-3-methylglutaryl-CoA (HMG CoA) reductase which regulates cholesterol biosynthesis in liver and other organs. Statins (e.g. lovastatin or pravastatin) are generally prescribed for the treatment of hypercholesterolemia. Apart from the cholesterol-lowering effect, statins exhibit “pleiotropic” effects such as anti-inflammatory effects, anti-thrombotic and anti-angiogenic effects, anti-hypertrophic effects, and plaque modifying effects (20-22). There is increasing evidence for an ameliorative effect of statins in renal diseases (23,24). Many in vitro studies have demonstrated that statins modulate a variety of intracellular signaling pathways
involved in cell proliferation and inflammatory responses in mesangial and renal tubular cells (25-28). Furthermore, preclinical studies in different experimental disease models showed that treatment with statins inhibited glomerular and interstitial recruitment of macrophages and neutrophils, independent of their cholesterol-lowering effects (29-31). Also, in ischemia-reperfusion renal injury models, treatment with statins substantially improved the renal functions (32,33). These potential cholesterol-independent effects of statins are due to the inhibition of an early step in the cholesterol synthesis pathway: statins inhibit the synthesis of isoprenoids such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate that are important posttranscriptional lipid attachments for intracellular signaling molecules Rho GTPases (34). The role of the Rho pathway in renal fibrosis will be described in a later section of this review. Several combination approaches such as statins plus an ACE inhibitor, and even statins and ACE inhibitors and then combined with ARB have been tested in renal fibrosis models and resulted in synergistic effects (35,36).

Clinical studies have also illustrated that long-term treatment with cerivastatin and pravastatin significantly reduced proteinuria in patients with IgA nephropathy and non-IgA nephropathy (37,38). In contrast, a recent study showed that fluvastatin treatment did not improve renal parameters, despite a positive effect on lipid parameters (39).

A few studies have demonstrated the therapeutic effects of Ang II blocking agents and statins on renal diseases in clinics. However, clinical studies with an apparent therapeutic effect on preventing end-stage renal failure have not been reported.

**New Therapeutic Approaches**

**Signaling cascade modifiers**

In recent years, the understanding of signal transduction cascades that play a role in the pathogenesis of renal diseases has increased and many potent signal transduction modifiers have become available. The studies presently available to date have been performed in experimental animal models for renal fibrosis and, no clinical data are available yet. Table 2 summarizes the studies carried out with different signal inhibitors in renal disease animal models. They represented an emerging research topic and may provide new leads for antifibrotic drugs.

**Mitogen– activated protein kinase and their inhibitors**

Four different subgroups of MAPK have been described which include (1) p38 MAPK, (2) extracellular signal-regulated kinases (ERKs), (3) c-jun N-terminal or stress-activated protein kinase (JNK/SAPK), and (4) ERK5. These MAPKs are activated by their specific upstream activators MAPK kinase (MKK) and a further activator MAPK kinase kinase (MKKK). For instance, p38 is activated by MKK3 and MKK6, and ERK1/2 is activated by both MAPK/ERK kinase (MEK)1 and MEK2 whereas JNKs are activated by MKK4 and
MKK7 (40). The role of MAPKs in renal fibrosis will be described here in more detail and several studies will be discussed.

**P38 MAPK and its inhibitors**

The p38 kinase is a ser/thr kinase and is activated by phosphorylation on Thr and Tyr residues in a Thr-Gly-Tyr motif by dual specific MKKs (41). There are four different p38 isoforms: p38α, p38β, p38γ and p38δ. P38α plays an important role in inflammation and is involved in the expression of IL-1β and TNF-α whereas the role of other isoforms is not well understood yet (42). Various stimuli, such as growth factors (Ang II, TGF-β), cytokines (IL-1β, TNF-α), stress factors (LPS, osmotic stress) and high glucose activate p38 in tubular and mesangial cells (Table 1, Fig. 2). The p38 pathway regulates the production of various proinflammatory genes (e.g. MCP-1, IL-1β, TNF-α and IL-6), and is involved in the expression of extracellular matrix components, intracellular enzymes and adhesion molecules. P38 is also associated with cell hypertrophy and cell differentiation (43). *In vivo* studies reveal that activation of p38, among other factors, is responsible for renal damage after hemorrhagic shock, unilateral ureteral obstruction, and ischemia injury in rats (44-46). Activation (phosphorylation) of p38 was also detected in tubular cells, glomerular endothelial cells and macrophages during tubulointerstitial injury (45,47). Moreover, in acute and chronic glomerulonephritis, the activation of p38 suggests an important role of p38 MAPK in the regulation of acute and chronic inflammation (48).

P38 MAPK inhibitors have been the most widely studied among all MAPK inhibitors, since p38 is activated by many important stimuli that play a role in renal disease, as described in Table 1. P38 regulates the production of TNF-α and IL-1 and, therefore, p38 inhibitors are supposed to interfere with inflammatory and immunoresponsive diseases (49). Archetypal p38 inhibitors are the derivatives of pyridinyl-imidazole such as SB-202190 and SB-203580. In recent years many new compounds have been developed, e.g. L-167307, AMG-548, BIRB-796, VX-745 and acyclic urea analogs (42,50). Fig. 3 depicts the chemical structure of some of these p38 inhibitors. All the above mentioned compounds competitively bind to the ATP−binding pocket of p38 kinase (51,52) and inhibit the phosphorylation of p38 and/or downstream transcription factors. The effect of many more p38 inhibitors for the treatment of renal inflammation and fibrosis has been studied in different animal models. Furuichi *et al* showed that treatment of renal ischemic mice with the p38 inhibitor FR167653 inhibited cell infiltration into the outer medulla and attenuated the extent of acute tubular necrosis (53). In addition, it decreased TNF−α, IL−1β, RANTES and MCP−1 gene expressions in ischemic kidneys. FR167653 has also been reported to reduce renal inflammation after burn injury (54). In both studies, FR167653 inhibited the phosphorylation of p38 in kidneys. In acute inflammatory renal injury either induced by cisplatin or in the anti-GBM glomerulonephritis model, p38 inhibitors significantly improved the renal functions (55,56). In the UUO rat model for renal fibrosis, blockade of p38α using its specific inhibitor NPC31169 markedly reduced ECM production (45). These
studies provide sufficient evidence that p38 inhibitors may be a valuable asset during the treatment of renal fibrosis.

Table 2. Overview of signal transduction modifiers evaluated in animal models for renal fibrosis.

<table>
<thead>
<tr>
<th>Category</th>
<th>Compound</th>
<th>Dose</th>
<th>Disease model</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>P38 MAPK inhibitors</td>
<td>FR167653</td>
<td>32 mg/kg/d, s.c.</td>
<td>renal ischemia-reperfusion mice model</td>
<td>(53)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 mg/kg/d, s.c.</td>
<td>Renal failure with HRP–induced burn injury</td>
<td>(54)</td>
</tr>
<tr>
<td></td>
<td>NPC31169</td>
<td>40 mg/kg b.i.d., gavage</td>
<td>anti-GBM glomerulonephritis rat model</td>
<td>(55)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40 mg/kg b.i.d., gavage</td>
<td>UUO rat model</td>
<td>(45)</td>
</tr>
<tr>
<td></td>
<td>SKF-86002</td>
<td></td>
<td>cisplatin-induced acute renal injury in mice</td>
<td>(56)</td>
</tr>
<tr>
<td>ERK inhibitors</td>
<td>U0126</td>
<td>10 mg/kg, i.v.</td>
<td>cisplatin-induced renal injury in mice</td>
<td>(63)</td>
</tr>
<tr>
<td>JNK inhibitor</td>
<td>CC-401</td>
<td>100 mg/kg, b.i.d., gavage</td>
<td>UUO rat model</td>
<td>(64)</td>
</tr>
<tr>
<td>Rho-ROCK inhibitors</td>
<td>Y-27632</td>
<td>40 mg/kg/d, p.o.</td>
<td>UUO mice model</td>
<td>(71)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 µg/kg, i.p.</td>
<td>Ischemia-reperfusion-induced acute renal failure in rats</td>
<td>(74)</td>
</tr>
<tr>
<td></td>
<td>Fasudil</td>
<td>10 mg/kg, i.p.</td>
<td>UUO rat model</td>
<td>(75)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 mg/kg/d, p.o.</td>
<td>Glomerulosclerosis in Dahl salt-</td>
<td>(76)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 mg/kg/d, i.p.</td>
<td>sensitive rats</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>spontaneously hypertensive rats</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>spontaneously hypertensive rats</td>
<td></td>
</tr>
<tr>
<td>NF-κB inhibitors</td>
<td>PDTC</td>
<td>50 mg/kg, b.i.d., i.p.</td>
<td>adriamycin–induced tubulointerstitial injury</td>
<td>(139)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200 mg/kg/d, p.o.</td>
<td>Passive Heymann nephritis rat model</td>
<td>(141)</td>
</tr>
<tr>
<td></td>
<td>Dehydroxymethyl-epoxyquinomycin</td>
<td>8 mg/kg/d, i.p.</td>
<td>UUO rat model</td>
<td>(143)</td>
</tr>
<tr>
<td></td>
<td>N-benzyloxy-carbonyl-Ile-Glu(o-t-Bu)-Ala-Leucinal</td>
<td>3 mg/kg/d, b.i.d., s.c.</td>
<td>UUO rat model</td>
<td>(153)</td>
</tr>
<tr>
<td>TGF-β receptor kinase inhibitor</td>
<td>SB-525334</td>
<td>10 mg/kg/d, p.o.</td>
<td>PAN-induced renal fibrosis</td>
<td>(105)</td>
</tr>
<tr>
<td>PDGF receptor kinase inhibitor</td>
<td>AG1295</td>
<td>12 mg/kg/d, i.p.</td>
<td>UUO rat model</td>
<td>(130)</td>
</tr>
<tr>
<td></td>
<td>STI571 (Gleevec)</td>
<td>50 mg/kg/d, i.p.</td>
<td>anti-Thy 1.1 glomerulonephritis</td>
<td>(132)</td>
</tr>
</tbody>
</table>

s.c., subcutaneously; i.p., intraperitoneally; p.o., orally
Several p38 inhibitors such as PS540446 (Pharmacopeia), Ro3201195 (Roche) and 8565533 (GlaxoSmithKline) are currently in clinical Phase I for the treatment of rheumatoid arthritis. Moreover, TAK-715 (Takeda) has advanced into clinical Phase II trials (57).

**ERK and its inhibitors**

ERK is a ser/thr kinase and is generally activated by both receptor tyrosine kinases and G-protein coupled receptors. ERK1 (p44) and ERK2 (p42) are activated by phosphorylation at the TEY motif by MEK1 and MEK2 which are in turn activated by the Ras/Raf pathway as depicted in Fig. 2 (40). In kidneys, a large number of stimuli including albumin, high glucose, angiotensin–II and cytokines, can activate the ERK pathway in tubular cells. These factors are strongly associated with the induction of renal diseases. Recent studies have demonstrated that ERK is activated during tubulointerstitial fibrosis induced by UUO or puromycin aminonucleoside (PAN) administration in animals (47,58). In addition, activation of the ERK pathway in the kidney has been correlated with increased cell proliferation, histologic lesions and renal dysfunction in human glomerulopathies (59). More evidence for activation of the ERK pathway has been reported during renal ischemia–reperfusion injury, in stroke-prone spontaneously hypertensive rats, in aldosterone/salt induced hypertensive rats and in Dahl salt-sensitive rat models (60-62).

Several potent MEK inhibitors such as U0126 (Fig. 2), PD98059, L-783277, RWJ-68354 have been developed to block ERK activity and attained to the preclinical development. Other MEK inhibitors CI-1040 and ARRY-142886 have reached phase-I stages of clinical trials in cancer patients (57). Jo et al showed that pretreatment of mice with the MEK inhibitor U0126 decreased ERK1/2 phosphorylation, improved renal functions and reduced TNF-α expression, apoptosis and leukocyte infiltration in the kidneys (63). This suggests that inhibition of ERK pathway can be a future approach for the treatment of renal disease.

**JNK and its inhibitors**

JNK pathway, also known as the stress activated protein kinase (SAPK) pathway, is generally activated by hypertonicity, heat shock and proinflammatory cytokines (Fig. 2). Members of the JNK pathway include JNK1 (or p46) and JNK2 (or p54) and brain specific JNK3 (p49) (40). MKK activates JNKs at their TPY motifs by dual phosphorylation. JNKs have been reported to play a role in inflammation, apoptosis and tumor formation. Activation of JNK in kidney disorders is found in mesengial and proximal tubular cells at an early stage of renal injury in PAN-induced renal disease (47). In addition, the JNK pathway has also been reported to be activated in podocytes, endothelial cells, macrophages and myofibroblasts in the crescent glomerulonephritis model (48). Treatment with the JNK inhibitor CC-401 in the UUO rat model reduced JNK phosphorylation which was associated with a reduced tubulointerstitial volume (64).
in alpha-smooth muscle actin and collagen-IV immunostaining and gene expression of TGF-β and CTGF was found after administration of CC-401.

The above mentioned studies reveal that p38, ERK and JNK pathways play a crucial role in the regulation of progression of renal diseases and those MAPK inhibitors may provide potential therapies for the treatment of renal fibrosis.

**Rho-ROCK system and its inhibitors**

Rho GTPases belong to the Ras superfamily of GTP–binding proteins. At least ten different Rho GTPases have been identified; Rho (A-E, and G), Rac and Cdc42 were studied most extensively. These GTPases switch between GTP-bound (active) and GDP-bound (inactive) conformations. Active (GTP-bound) Rho interacts with effector molecules such as Rho–kinases (ROCK-I and ROCK-II), mDia, Rhophilin, Rhotekin, and protein kinase N which initiate downstream signaling cascade (65). An important role for Rho in the etiology of renal diseases has been acknowledged recently (66). Several profibrogenic growth factors such as Ang II, lysophosphatidic acid, TGF-β, PDGF, and endothelin-I have been reported to activate Rho-dependent pathways (67-70). The activation of the Rho-ROCK system is shown in Fig. 2. Activation of human renal fibroblasts with TGF-β caused induction of connective tissue growth factor (CTGF), which is a profibrotic factor and this effect was abrogated by RhoA specific inhibitors (68). Inhibition of Rho-ROCK system also suppressed migration of macrophages but could not inhibit proliferation of renal fibroblasts (71). Epithelial mesenchymal transdifferentiation, a key process in tubulointerstitial fibrosis, was blocked by Rho/Rho kinase pathway inhibition in human renal tubular cells (72). These studies demonstrate that Rho signaling may play a crucial role in the pathogenesis of renal fibrosis.

Y-27632 and fasudil are specific inhibitors of ROCK (chemical structures are shown in Fig. 3). Y-27632 inhibits ROCK by binding to the catalytic site with 10-20 fold higher affinity towards ROCK-I/II in comparison to other Rho kinases. It is metabolized rapidly in vivo and does not cause major side effects even at higher doses (73). In the UUO model of renal fibrosis, specific inhibition of ROCK with Y-27632 decreased smooth muscle actin, TGF-β and collagen expression, macrophage infiltration and interstitial fibrosis (71). Treatment with Y-27632 at a low dose of 100µg/kg/d i.p. for 2 weeks prevented the development of ischemia/reperfusion-induced acute renal failure (74), supporting a pivotal role of ROCK in this process.

Fasudil (or HA-1077) inhibits ROCK by binding to its catalytic site. It is metabolized rapidly to its active metabolite hydroxyfasudil which inhibits Rho-kinase with a Ki similar to the parent compound (73). Fasudil inhibited monocyte and macrophages infiltration and interstitial fibrosis in UUO rat model (75). Treatment with fasudil in Dahl salt-sensitive rats improved renal functions and attenuated glomerulosclerosis by reducing TGF-β and collagen expression (76). In subtotally nephrectomized spontaneously hypertensive rats,
fasudil inhibited tubular injury by upregulating \( p27 \) and subsequently inhibited cell proliferation and macrophage recruitment (77).

**Transforming growth factor-\( \beta \) and its inhibitors.**

The TGF-\( \beta \) superfamily includes three TGF-\( \beta \) isoforms (TGF-\( \beta 1, \beta 2, \) and \( \beta 3 \)), activins, inhibins, and bone morphogenic protein (BMP). TGF-\( \beta \) is considered a multifunctional cytokine that can regulate cell proliferation, differentiation, immune response, apoptosis and extracellular matrix deposition (78,79). Most of the intracellular signaling through TGF-\( \beta \) is mediated via Smad pathways which are activated/phosphorylated by TGF-\( \beta \) type I receptors (ALK5, ALK1 and ALK8). However, also Smad-independent pathways such as MAPK, NF-kB or PI3 kinase/AKT pathways are activated by TGF-\( \beta \) (80-82) as illustrated in Fig. 2. TGF-\( \beta \) signaling is considered to be a key regulator of the extracellular matrix deposition during renal fibrosis. TGF-\( \beta \) expression can be produced by different cell types within the kidney depending on the type of renal injury. TGF-\( \beta \), in turn, can stimulate mesangial cells, interstitial fibroblasts, and tubular epithelial cells in vitro to undergo myofibroblastic activation or transition and to become ECM producing fibrogenic cells (83). Following UUO in animals, TGF-\( \beta \) is increased significantly in proximal tubules, thick ascending limbs of Henle, and distal convoluted tubules, whereas its expression in glomeruli and collecting ducts was constant (84,85). It is likely that TGF-\( \beta \) is also produced by macrophages since a strong correlation has been found between TGF-\( \beta \) and numbers of macrophages (86). In PAN, gentamycin and adriamycin nephritis models, TGF-\( \beta \) expression also increases significantly in the renal cortex demonstrating the apparent role of TGF-\( \beta \) in renal fibrosis (87-89).

In patients with glomerulonephritis and in experimental tubulointerstitial fibrosis models like unilateral ureteral obstruction, apoptosis in tubular epithelial cells produces tubular atrophy (90,91). In these studies, a correlation between TGF-\( \beta 1 \) expression and apoptosis in tubular cells was observed, implicating the proapoptotic role of this growth factor in renal diseases. Studies in a TGF-\( \beta 1 \) transgenic model of glomerulosclerosis have indicated that TGF-\( \beta 1 \) causes apoptosis in podocytes and in glomerular endothelial cells (92,93). A recent study has reported elevated levels of plasma TGF-\( \beta \) in patients with mild to moderate ureteral obstruction (94). In addition, a substantial correlation was found between glomerular TGF-\( \beta 1 \) expression levels and severity of glomerulosclerosis (95).

For many years, therapeutic approaches aiming at a TGF-\( \beta \) blockade have been pursued in order to prevent the progression of renal fibrosis. These include antisense oligonucleotide of TGF-\( \beta \), neutralizing anti-TGF-\( \beta \) antibody and blockade of TGF-\( \beta \) activation by decorin or soluble TGF-\( \beta \) receptor (83). Other indirect approaches have been used to inhibit TGF-\( \beta \) signaling like the use of Ang II receptor antagonists or antisense inhibition of CTGF in animal renal fibrosis models (96-99). A more direct inhibition of TGF-\( \beta \) signaling can be effectuated via inhibition of the TGF-\( \beta \) type I receptor kinase, also
known as the Activin Receptor Like Kinase (ALK5). ALK5 phosphorylates Smad 2 and 3, which mediate profibrotic effects of TGF–β1 for instance tubular dedifferentiation and the deposition of ECM. The inhibitors of ALK5 belong to dihydropyrroloimidazole and tiarylimidazole analogues which interact with the ATP-binding site of ALK5 (100,101).

Figure 2. Different signaling pathways involved in the tubulointerstitial fibrosis.

The p38 inhibitor SB203580 also inhibited the ALK5 phosphorylation of Smad3 due to the similarities between the ATP-binding pocket of ALK5 and serine/threonine kinase p38 (101). Recently, the potent and specific ALK5 inhibitor SB-431542 was developed which had no effect on any other signaling pathway but clearly inhibited TGF–β1-induced procollagen-1αI expression in renal epithelial cells (100,102,103). Procollagen-1αI is an important marker for increased ECM deposition, so SB-43154 may be quite relevant. Thereafter, another pyridinyl imidazole compound SB-505124 was developed which inhibited ALK4-, ALK5-, and ALK7-dependent activation of Smad2 and Smad3 selectively (104). This compound blocked the effects of TGF–β in prostrate epithelial cells and hepatoma cells (104). In the same series of compounds, SB-525334 was found to be
effective for the treatment of renal progressive diseases. SB-525334 inhibited TGF-β induced phosphorylation of Smad2 and Smad3 and expression of PAI-1 and procollagen-1αI in renal epithelial carcinoma cells (105). In this study, treatment with SB-525334 during acute PAN-induced renal fibrosis in rats reduced the fibrotic markers procollagen-1αI and -1αIII significantly. Recently, A-83-01 was found to be more potent in the inhibition of ALK5 than SB-431542, and also inhibited the epithelial-to-mesenchymal transition induced by TGF-β (106). SD-208, a selective and novel 2,4-disubstituted pteridine derivative blocked TGF-β-induced PAI-1 expression in vitro and retarded the progression of established lung fibrosis in rats (107). The new ALK5 inhibitor GW6604 was shown to inhibit the process of liver fibrosis (108). In vitro, GW6604 inhibited the expression of liver fibrosis markers in hepatocytes and in vivo reduced collagen expression and matrix deposition in dimethylnitrosamine–induced liver fibrosis model. These studies reveal that ALK5 inhibitors are effective in vivo and can be the new potential therapeutics for renal fibrosis.

**Platelet derived growth factor (PDGF) and its inhibitors**

PDGF is a potent growth factor for mesenchymal cell types such as myofibroblasts. The PDGF family is composed of PDGF−A, −B, −C and −D, which form either the homodimers or heterodimers PDGF−AA, −AB, −BB, −CC, and −DD. These isoforms exert their cellular effect by binding to the structurally similar tyrosine kinase receptors −α and −β. These receptors, in turn, dimerize when the dimeric PDGF binds and create either homodimers or heterodimers of the receptors in three combinations PDGFR−αα, −αβ and −ββ. PDGF−AA, −AB, −BB and −CC can induce αα receptor homodimers, PDGF−AB, −BB can induce αβ form and PDGF−BB and −DD can induce ββ receptor form (109,110). PDGFs play pivotal roles in wound healing, regulation of interstitial fluid pressure and embryonic development. In addition, they participate in malignancies, atherosclerosis and fibrotic diseases (109,111). The role of PDGF in various human and animal renal diseases has been confirmed by the increased expression of PDGF and PDGF receptors in renal tissue. The induced expression of PDGF and its receptor was found in patients with IgA nephropathy and other human proliferative glomerulonephritis (112-114). PDGF-BB has been recognized as a potent mitogen and chemoattractant for renal mesangial cells and also induces ECM deposition, which leads to glomerulosclerosis (111). Iida et al found a marked increase in PDGF−A and −B and PDGFR−β mRNA expression in glomeruli in the mesangial proliferative nephritis model in rats (115). The important role of PDGF in tubulointerstitial fibrosis was demonstrated when administration of PDGF-BB in rats substantially increased tubulointerstitial cell proliferation and expressions of alpha-smooth muscle actin and collagens (116). In human diabetic nephropathy, gene expression levels of PDGF−A and −B were found to be several fold increased and immunohistochemistry showed that PDGF−A was present at both glomeruli and proximal tubular cells whereas PDGF−B was localized in the areas of peritubular, interstitial and periglomerular fibrosis.
The functions of the new members of the PDGF family, PDGF-C and PDGF-D have been also reported recently in renal diseases (118,119). PDGF-C is constitutively expressed in parietal Bowman’s capsule, tubular epithelial cells and arterial endothelial cells and upregulated in podocytes and interstitial cells after renal injury (118). PDGF-D was found to be normally expressed in vascular smooth muscle and mesangial cells but not in other interstitial cells. However, PDGF-D was highly expressed in interstitial cells, mainly in myofibroblasts and other fibrotic areas in the UUO mice model as well as in mesangial cells in the anti-Thy 1.1 glomerulonephritis model (119,120).

Inhibition of PDGF-mediated processes using different approaches has been employed for the treatment of fibrosis. However there is little evidence for an effective treatment of renal fibrosis (121-123). Pirfenidone, an antifibrotic drug, reduced lung fibrosis probably by inhibiting the production of PDGF–A and –B isoforms by lung macrophages (121). Application of soluble PDGF-β receptor to influence proliferative hepatic stellate cells caused inhibition of their proliferation and, even more important, its intravenous administration to the bile duct ligated animals significantly reduced the expression of collagen and alpha-smooth muscle actin (123). Trapidil, an antagonist of the PDGF receptor, reduced mesangial cell proliferation induced by different growth factors in vitro and in vivo in anti-thymocyte serum–induced glomerulonephritis model in rats (124,125). Treatment with trapidil reduced the nephrotoxicity of gentamicin in rats (126). In contrast, administration of trapidil in renal ischemic rats worsened the renal damage probably by inhibiting the tubular repair process after acute tubular injury (127), induced by PDGF.

A variety of chemical compounds have been developed as selective PDGFR kinase inhibitors and applied for the treatment of fibrosis. Chemical structures of some of these inhibitors are shown in Fig. 3. Quinoxalines such as AG1295, AG1296, AGL 2033 and AGL 2043 have been reported to be highly potent and selective towards PDGFR (128). They bind to the ATP binding site of the tyrosine kinases. All these inhibitors contain an aryl group that interacts with a lipophilic pocket near the ATP binding site, not available to ATP. This extra binding is probably essential for the potency of these inhibitors and seems to be an important factor in their selectivity (128). These compounds were proven to reduce fibrosis in animal models. In a rat model of pulmonary fibrosis, administration of AG1296 reduced the number of proliferative epithelial and mesenchymal cells by 50% (122). In vitro, AG1295 treatment effectively decreased PDGF-BB–induced proliferation of hepatic stellate cells and also inhibited autophosphorylation of PDGFR–β. PDGF-BB–induced activation of MAPK isoforms and tyrosine phosphorylation of PI3-kinase, PLC-γ and p21 ras guanosine triphosphate-activating protein in these cells (129). Interestingly, treatment with AG1295 substantially reduced interstitial fibrosis in the rat UUO model by reducing the tubulointerstitial area, ECM deposition and number of macrophages (130).

Another category of PDGFR tyrosine kinase inhibitors are phenylaminopyramidines, represented by ST1571/ imatinib/Gleevec which also blocks Bcr-Abl kinase, indicating its different mode of binding as compared to the other compounds (131). Clinical use of
Gleevec has been approved so far only for secluded cases of chronic myelomonocytic leukemia and dermatofibrosarcoma protuberans with very good response (131). Gleevec reduced PDGF–stimulated mesangial cells proliferation in vitro and the number of alpha-smooth muscle actin positive mesangial cells as well as glomerular collagen deposition in animals with anti-Thy 1.1 glomerulonephritis (132). In a recent study, treatment with three distinct PDGFR tyrosine kinase inhibitors SU9518, SU11657 or Gleevec markedly attenuated the development of irradiation–induced pulmonary fibrosis in mice (133). Upon irradiation, up-regulated expression of PDGF (A-D) isoforms led to phosphorylation of PDGF receptors and this was highly inhibited by these inhibitors.

**Nuclear factor (NF)-κB signaling and its inhibitors**

NF-κB comprises a family of transcription factors that play a crucial role in the regulation of innate and adaptive immunity. The NF-κB family is composed of five members p65 (RelA), RelB, c-Rel, p50/p105 (NF-κB1) and p52/p100 (NF-κB2). They exist as homo- and hetero–dimers bound to IκB family proteins in unstimulated cells. Upon activation, IκB proteins are phosphorylated by IκB kinases, IKKα and IKKβ, and ubiquitinated and degraded in proteasomes. NF-κB pathway can be activated by distinct signal transduction cascades such as TNF-α, IL-1, LPS or stress–mediated cascades (Fig. 2). Degradation of IκB allows NF-κB to translocate from cytoplasm into the nucleus where it binds to DNA to regulate the production of various cytokines, chemokines, stress response proteins and anti-apoptotic proteins (134,135). For further details on signaling of NF-κB we recommend to read a recent review by Hayden and Ghosh (136).

NF-κB is a main mediator of genes that regulate cell proliferation and apoptosis which makes NF-κB an important participant in the pathogenesis of cancer (135,137). NF-κB is also associated with the pathogenesis of chronic inflammatory diseases such as asthma, rheumatoid arthritis and inflammatory bowel disease, since it regulates the expression of proinflammatory cytokines, chemokines and adhesion molecules. The pathogenic significance of NF-κB pathway in renal fibrosis has been identified in recent years. The following studies provided strong evidence for the involvement of NF-κB activation in various experimental animal models for renal diseases. Sakurai *et al* showed that induction of glomerulonephritis with nephrotoxic serum in rats caused NF-κB activation in glomeruli which was increased from day 3 to5 and persisted until day 14 (138). In adriamycin–induced tubulointerstitial injury, the activity of NF-κB (p50/65, p50/c-Rel) was increased in the renal cortex from day 7 and reached a maximum on day 28. This pattern was inhibited by chronic treatment with a specific NF-κB inhibitor (139). Garre *et al* showed the activation of NF-kB in proximal tubular cells in vivo in protein over-load renal fibrosis model and in vitro models in relation to Ang II and endothelin-1 in tubulointerstitial injury (140). Furthermore, in passive Heymann nephritis, NF-κB activation predominantly occurred in podocytes and correlated with the existence of proteinuria (141).
In many other animal models for tubulointerstitial fibrosis and glomerulonephritis, activation of NF-κB was found to be localized in tubular and glomerular cells, respectively (142-145). Sakai et al identified NF-κB-positive cells in patients with crescentic glomerulonephritis which were mainly present in crescentic lesions, tubular epithelial cells, and interstitial mononuclear infiltrates (146). Recently the same research group established the activation NF-κB as the p65-positive nuclei were found in kidneys of patients with diabetic nephropathy. These NF-κB (p65) positive cells were identified as mesengial cells, endothelial cells, podocytes, tubular cells, and mononuclear infiltrates in the interstitium (147). In both studies, they demonstrated that NF-κB activation was associated with the
phosphorylation of p38 MAP kinase in glomerular cells during glomerulonephritis and in tubulointerstitial cells during diabetic nephropathy.

NF-κB inhibition has drawn much attention in the field of drug development since it regulates various crucial pathophysiologic pathways. A wide range of NF-κB inhibitors have been developed during the last decade and, moreover, plenty of existing compounds have been identified as NF-κB inhibitors (for further details see review by Aggarwal et al) (148). In this review we will focus on those studies in which NF-κB inhibitors have been tested in renal fibrosis models. As an example, pyrrolidine dithiocarbamate (PDTC) has been examined in various renal disease models for its efficacy. Treatment with PDTC inhibited NF-κB activity and reduced tubular cells atrophy, interstitial volume, ED-1 infiltration and cortical lipid peroxidation without any effect on proteinuria in adriamycin-induced tubulointerstitial injury in rats (139). In the Passive Heymann nephritis rat model, PDTC markedly decreased MMP-9 mRNA levels in glomeruli and it also reduced the proteinuria (141). Moreover, in other renal disease models such as FK506-induced nephropathy and gentamicin-induced tubulointerstitial injury, PDTC treatment attenuated the disease in both cases (142,149). Glucocorticoids such as prednisolone and dexamethasone have also been reported to inhibit NF-κB activity in nephrotoxic serum-induced glomerulonephritis in rats and in rat mesangial cells in vitro, respectively (150,151). It has been suggested that glucocorticoids inhibit the NF-κB activity by increasing the levels of IκB resulting in cytoplasmic retention of p65 and/or by inhibiting NF-κB DNA binding (135). Lopez-Franco et al showed that treatment with different NF-κB inhibitors such as gliotoxin (a fungal metabolite) or parthenolide (a plant extract), significantly reduced proteinuria and glomerular damage in anti-Thy 1.1 rat model and anti-mesangial cell nephritis in mice, respectively (145). Miyajima et al evaluated a novel NF-κB inhibitor dehydroxymethyl-epoxyquinomicin (DHMEQ) in the UUO rat model and found a substantial reduction in apoptosis and proliferation of tubular cells, which was associated with reduced interstitial fibrosis (143). They showed that DHMEQ inhibited NF-κB activity by preventing p65 translocation to nuclei which had also been described by others (152). Since IκB is degraded in proteasomes enabling translocation of NF-κB to the nucleus, inhibition of this degradation is a relevant approach to inhibit NF-κB activity. Administration of the proteasome inhibitor N-benzyloxy-carbonyl-Ile-Glu(o-t-Bu)-Ala-Leucinal, to UUO rats decreased inflammation and attenuated the development of fibrosis by reducing MCP-1 gene expression, macrophages influx and gene expression of pro-fibrogenic molecules (153). These studies verify that if NF-κB activity is inhibited, significant reduction of renal disease is achieved in several cases.

Renal drug targeting

Many reasons can be portrayed to perform renal drug targeting. Various compounds used for the treatment of renal fibrosis do not sufficiently reach the kidneys to achieve therapeutic levels at tolerable doses. Even though compounds may reach the kidneys, they
might not enter the specific target cell aimed at due to suboptimal intra-renal transport. As a consequence, high doses are needed to achieve therapeutic levels in the kidneys which in turn cause extra-renal side effects. Additionally, pathological conditions and rapid (local) metabolism of the drugs can limit the renal distribution and/or accumulation of drugs at the target site. To deliver therapeutic amounts of drugs and to avoid interactions with non-target organs, renal targeting therefore may be an interesting approach.

Renal specific delivery of drugs can be achieved by carriers that are filtered in the glomerulus and reabsorbed from the urine by receptor-mediated endocytosis in the proximal tubule as shown in Fig. 4. Alternatively, carrier groups can be attached that facilitate uptake via receptors at the basolateral membrane of proximal tubular cells, thus not requiring the filtration of the compound into the urine (154,155). Low molecular weight proteins (LMWPs) and low molecular weight polymers belong to the first category of carriers, and are suitable for renal targeting for various reasons. First, when the size of these macromolecules allows glomerular filtration, they accumulate rapidly in the proximal tubular cells where, in principle, they can release free drug intracellularly. Of note, the filtration and subsequent accumulation of carriers may depend on the charge since negatively charged carriers are repelled by the glomerular basement membrane. This is important since charge and other physiochemical properties of the carrier can be modified after coupling the drugs to the carrier. Only a few studies have reported the renal delivery of drugs using polymers (156). In contrast, lysozyme has extensively been employed to deliver various drugs to the kidneys. Franssen et al described various drug-LMWP conjugates with different linkages between drug and protein (157). It has been established that drug-lysozyme conjugates are internalized by proximal tubular cells via the megalin receptor through an endocytotic mechanism. Inside the lysosomes, the protein carrier is degraded by proteolytical enzymes and the drug is released either enzymatically or by pH–sensitive linkage degradation. The released drug may produce its pharmacological effect inside the target cell but may also diffuse outside the cell and produce its action at the cell surface or in other cell types. In the latter case, the tubulointerstitial cell serves as a slow release depot for drugs within the kidneys.

The anti-inflammatory drug naproxen and the ACE inhibitor captopril have been targeted to the kidneys by coupling them to lysozyme using amide and disulfide linkages, respectively (158,159). These drug-lysozyme conjugates produced renal–specific effects after intravenous administration. Moreover, captopril-lysozyme conjugate could be administered through the subcutaneous route which provided a slow and prolonged accumulation of the conjugate in kidneys (160). Prolonged treatment of this captopril conjugate exhibited a significant reduction in proteinuria without affecting systolic blood pressure in adriamycin–induced nephrotic animals (161). Using the same renal carrier system, we have recently delivered the p38 MAPK inhibitor SB202190 to the kidneys (162). The SB202190-lysozyme efficiently accumulated in the kidneys and the drug was released from its carrier during a prolonged period of time, which is preferred for a chronic
drug effect. At present, we are investigating the antifibrotic effects of this conjugate, and drug-lysozyme conjugates with other kinase inhibitors are under development. This approach will allow local intervention in kinase pathways involved in the development of renal fibrosis, without the risk of side effects outside the kidneys. Many of the MAP kinase, ERK, JNK or other signaling inhibitors require local high concentrations within/around the target cells but distribute poorly to the kidneys. As a consequence, they may benefit greatly from renal targeting.

**Gene therapy**

In the contest of therapeutics for renal diseases, gene therapy has also shared its contribution. For renal gene therapy, several techniques such as viral, nonviral, and cellular vectors have been used both *in vivo* and *ex vivo* (163). Ideally, an efficient and selective gene delivery is needed to achieve therapeutic effects in kidneys and avoid interaction with other organs.

Wolf *et al* demonstrated that kallikrein adenoviral gene delivery in rats with 5/6 nephrectomy–induced fibrosis reduced the occurrence of glomerular sclerotic lesions, tubular damage, and interstitial inflammation in kidneys (164). Kallikrein cleaves kininogen substrate into kinin which plays a pivotal role in cardiovascular function. Furthermore, kallikrein gene therapy substantially decreased DOCA–salt induced proteinuria, glomerulosclerosis, tubular dilation, and luminal protein casts which resulted in a reduction of renal fibrosis (165). Adenovirus–mediated delivery of a vasodilator gene, adrenomedullin, attenuated renal damage in hypertensive dahl salt-sensitive rats and Goldblatt hypertensive rats (166,167). Adenoviral gene delivery has also been employed to deliver the entire soluble extradomain of the TGF-β type II receptor (fused with IgG) into the kidneys of rats with anti-GBM nephritis in order to block the action of TGF-β (168). To affect the TGF-β signaling, non-viral transfection techniques have also been used. A doxycycline-regulated Smad7 gene was delivered by renal arterial injection in rats with UUO or 5/6 nephrectomy using an ultrasound-microbubble (Optison)-mediated system. This inhibited tubulointerstitial fibrosis in terms of reduced myofibroblasts accumulation and collagen expression (169,170).

Since HGF displayed beneficial effects in renal fibrosis, gene delivery of HGF via systemic administration of naked plasmid vector was also tested. This treatment ameliorated renal fibrosis induced by UUO or streptozotocin–induced diabetic nephropathy in mice (171,172). Gene delivery of HGF was performed by various methods such as liposomes containing hemagglutinating virus of Japan (HVJ liposome) and by electroporation of plasmid vector encoding HGF (173,174). In these studies, gene therapy of HGF prevented the tubulointerstitial fibrosis induced by either UUO or cyclosporine. In another approach, intrarenal injection of the naked plasmid 7ND, encoding for an N-terminal deletion mutant of the human MCP-1 gene, significantly reduced macrophage infiltration, tubular damage, and gene expression of TGF-β1 and MCP-1 in the protein-
overload renal injury model (175). In the UUO mice model, gene delivery of 7ND produced beneficial effects on renal fibrosis (176).

Yokoi et al demonstrated that administration of CTGF antisense oligonucleotide by hydrodynamic-based gene transfer technique in rats with UUO attenuated the induction of fibronectin, ED-A and collagen Iα(I) gene expression as well as collagen deposition in the interstitial fibrotic area (177). Short synthetic interfering RNA duplexes (siRNAs) can selectively silence the expression of a complementary gene in mammalian cells. Recently this technique has been applied in different animal models of renal disease. siRNA molecular targeting against TGF-β1 were administered via the renal artery followed by electroporation in anti-Thy 1.1–induced glomerulonephritis in rats (178). This treatment significantly suppressed TGF-β1 mRNA and protein expression, thereby inhibiting the deposition of ECM. In another study, a plasmid DNA expressing siRNA against TGF-β receptor was injected into the kidney via the ureter and then UUO was performed. This

Figure 4. Schematic presentation of drug delivery and gene delivery to the kidneys.
resulted in reduced TGF-βR levels and alpha smooth muscle actin and collagen expression in the renal cortex (179).

There are many examples of renal gene delivery that have not been discussed here. These studies reveal that gene therapy using viral and non-viral approaches can be the future therapeutic interventions for renal diseases. At present, safety issues related to these techniques are major hurdles that need to be resolved before one can adopt these techniques in patients.

Other therapeutic interventions

Recently identified biological therapeutics includes Interleukin-10 (IL-10), bone morphogenetic protein-7 (BMP-7), hepatocyte growth factor, and transglutaminase inhibitors. IL-10 is an immunomodulatory cytokine produced by monocytes/macrophages, B cells, and T cells. IL-10 acts as an endogenous immunosuppressive and anti-inflammatory factor during inflammation. Administration of IL-10 to mice with glomerulonephritis induced by anti-GBM globulins prevented the decline in renal function and markedly diminished glomerular T cell and macrophage accumulation (180). Further studies confirmed that IL-10 administration attenuated glomerulonephritis induced by either anti-GBM or anti-Thy 1.1 antibody (181,182). A recent study showed that treatment with another interleukin (IL-11) reduced glomerular NF-κB activity markedly, caused a reduction in glomerular macrophage infiltration, fibrin deposition and albuminuria in nephrotoxic serum induced glomerulonephritis in mice (183). BMPs are ligands for ALK receptors and phosphorylate Smad-1, -5 and -8. BMP-7 has been found to be reduced during acute renal injury in an ischemia mouse model (184,185). The therapeutic potential of BMP-7 has been recently reviewed (186). Briefly, administration of recombinant BMP-7 in the UUO model inhibited interstitial inflammation, fibrogenesis, apoptosis and tubular atrophy (187,188). Similarly, treatment with recombinant BMP-7 improved renal functions and reduced interstitial fibrosis in two genetic mouse models (189).

HGF is an endogenous peptide, composed of a 69 KDa α-chain and a 34 KDa beta-chain and antagonizes the effects of TGF-β. HGF binds to its specific tyrosine kinase receptor c-met, thereby stimulating cell proliferation and differentiation and also cell migration and tumorigenesis. Liu has described the therapeutic effects of HGF in renal fibrosis in detail (190). In short, administration of recombinant HGF into UUO mice substantially suppressed the progression of renal interstitial fibrosis with a decrease in renal alpha-smooth muscle actin expression, total collagen content, interstitial matrix components such as fibronectin, and renal expression of TGF-β1 and its type I receptor (191). Moreover, in the rat remnant kidney model, a continuous infusion of HGF prevented tubulointerstitial fibrosis and collagen deposition which was associated with increased tubular expression of MMP-9 (192).
Stabilization of the ECM has been considered as an important mechanism to prevent the progression of renal fibrosis. Tissue transglutaminase is a calcium-dependent enzyme that contributes to the stabilization of ECM proteins by forming \( \gamma \)-glutamyl-lysine cross-links. In subtotal nephrectomy and diabetic nephropathy animal models, activation of tissue transglutaminase was found within tubular cells and glomeruli (193,194). An highly specific site–directed inhibitor of tissue transglutaminase (1,3-dimethyl-2[(oxopropyl)thio]imidazolium) inhibited the glucose-induced deposition of ECM proteins in renal tubular epithelial cells in vitro (195). If further evidence for in vivo effectiveness can be obtained, transglutaminase inhibitors can open a future direction for the treatment of renal fibrosis.

Conclusions

Tubulointerstitial fibrosis is a complex process involving various factors associated with diverse intracellular signaling cascades. Therefore, signal transduction therapies are currently taking the attention as future therapeutic interventions complementary to the current therapies. Yet, efficacies of these therapies have been definitely verified only in experimental animal models of renal fibrosis. Most of the clinical studies performed at present with the candidate compounds have been carried out for rheumatoid arthritis, cancer and other diseases but not yet for renal fibrosis. This invites further clinical evaluation of the particular concepts in which long term toxicity aspects will be included. In view of the multiple and complex mechanisms underlying chronic renal diseases, the question should be addressed if combination therapies are rather necessary to cope with the multifactorial pathological process. Classical treatment schemes may be combined with the novel approaches mentioned in the present review. New experimental therapies may reveal whether the strategy to inhibit one of the signal transduction pathways outlined in this review will provide an effective therapy for this chronic disease. It remains to be established whether interference in this complete system of signal amplification provides powerful tools to treat this disease, creates serious adverse effects due to the ubiquitous presence of the signal pathways or that redundancy within the system leads to a dampening of all the pharmacological effects of drugs. In any case, new opportunities and questions emerge to resolve the complexity of renal fibrosis.

Acknowledgements

This work was made possible by a grant from SenterNovem (TSGE1083).

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Novel therapeutic targets for the treatment of renal fibrosis


Chapter 4


Novel therapeutic targets for the treatment of renal fibrosis


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Novel therapeutic targets for the treatment of renal fibrosis


