SMP-534 inhibits TGF-β-induced ECM production in fibroblast cells and reduces mesangial matrix accumulation in experimental glomerulonephritis

Eiji Sugaru,1 Mutsuko Sakai,1 Kazuhiko Horigome, Teruhisa Tokunaga,3 Makoto Kito,3 W. Ewan Hume,3 Ryu Nagata,3 Tsutomu Nakagawa,1 and Mutsuo Taiji1

1Discovery Research Laboratories I, 2Genomic Research Laboratories, and 3Chemical Research Laboratories, Sumitomo Pharmaceuticals Research Division, Osaka, Japan

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Sugaru, Eiji, Mutsuko Sakai, Kazuhiko Horigome, Teruhisa Tokunaga, Makoto Kito, W. Ewan Hume, Ryu Nagata, Tsutomu Nakagawa, and Mutsuo Taiji. SMP-534 inhibits TGF-β-induced ECM production in fibroblast cells and reduces mesangial matrix accumulation in experimental glomerulonephritis. Am J Physiol Renal Physiol 289: F998–F1004, 2005. First published May 17, 2005; doi:10.1152/ajprenal.00065.2005.—Transforming growth factor-β (TGF-β) is a potent fibrotic factor responsible for the synthesis of extracellular matrix (ECM) and is implicated as the major determinant in pathogenesis of chronic fibroses, including kidney. The novel small compound SMP-534 reduced ECM production induced by TGF-β in fibroblast cells. SMP-534 inhibited TGF-β-induced p38 mitogen-activated protein kinase (p38) activation but did not inhibit epidermal growth factor (EGF)-induced extracellular signal-related kinase (ERK) activation. We also found that oral administration of SMP-534 dose dependently lowered hydroxyproline contents in the cortical region of the kidney in rat anti-Thy-1 nephritis models. In periodic acid-Schiff staining of kidney sections, ECM accumulation was reduced by SMP-534 treatment. These data indicate that SMP-534 has potential in therapy for fibrotic diseases, including nephropathy.

renal fibrosis; anti-Thy-1 nephritis; proteoglycan; p38 mitogen-activated protein kinase

PROGRESSIVE RENAL FUNCTION loss associated with chronic renal diseases can be produced either by the development of glomerulosclerosis or by progressive tubular interstitial fibrosis. Renal fibrogenesis can be induced by several mechanisms in different renal diseases but will finally produce identical fibrotic changes in the kidney. Tissue fibrosis is the result of excessive accumulation of extracellular matrix (ECM) that, in the kidney, impairs renal function and finally leads to organ failure (10).

Transforming growth factor-β (TGF-β) promotes the accumulation of ECM by increasing expression of ECM genes, such as collagen and proteoglycan (3), and by inhibiting the gene expression responsible for degradation of ECM (24). An extensive number of studies indicate that TGF-β plays an important role in renal fibrosis (4). Thus inhibition of ECM production induced by TGF-β is a potentially useful means for treatment of renal fibrosis. Fibrosis is alleviated by administering TGF-β antiserum or antibody in animal models (6, 25, 35). However, when we consider compliance to patients, an orally active molecule is preferable. Therefore, a small molecule that can inhibit TGF-β action and reduce ECM production is expected to be a good therapeutic medicine against tissue fibrosis.

As reviewed earlier (21, 26), a TGF-β receptor ligand initiates signaling by binding to and bringing together type I [activin receptor-like kinases 5 (ALK5)] and type II receptor kinases on the cell surface. This allows receptor II to activate ALK5, which then propagates the signal through phosphorylation of Smad2/3. Laping's group (17, 20) reported a very interesting molecule, SB-431542, which selectively inhibits ALK5 kinase. SB-431542 prevented elevation of fibronectin, plasminogen activator inhibitor 1, and collagen mRNA synthesis triggered by TGF-β (17, 20). One place to look for agents to block TGF-β action is downstream from the TGF-β receptor. Several signaling molecules are possible targets. For example, Smad2 and Smad3, which are directly phosphorylated and activated by ALK5, undergo homotrimerization and formation of heteromeric complexes with Smad4. Activated Smad complexes are translocated into the nucleus and regulate transcription of target genes, including ECM. In addition to the Smad group of proteins, another pathway involving a member of the mitogen-activated protein kinase kinase (MAPK) family of kinases, TGF-β-activated kinase (TAK1), is also involved in TGF-β signaling. TGF-β-activated kinase phosphorylates and activates p38 through MAP kinase kinase (MKK) 6 and MKK3 (15, 29). As demonstrated by Varela-Rey et al. (28), p38 activation induced by TGF-β also has an important role in collagen production. Several TGF-β inhibitors have been reported, among which SB-431542, as mentioned above, inhibits ALK5 directly. In contrast, other antifibrotic agents show inhibitory profiles against TGF-β actions in cultured cells or animal models, but their mechanisms are not clear (11). Therefore, it was intriguing for us to see whether we could get one of the original molecules to reduce ECM production induced by TGF-β. To identify inhibitors of ECM production induced by TGF-β, a cell-based assay was developed. Screening of our internal compound collection resulted in identification of lead compounds. Among several substituted pyrrol derivatives that we chemically synthesized and optimized from lead compounds, we finally obtained 5-chloro-2-{[(1E,3)-2-(4-methoxybenzoyl)-4-methyl-1H-pyrrol-1-yl)prop-1-en-1-yl]-N-(methylsulfonyl)benzamide (SMP-534). To determine whether SMP-534 is a potentially useful therapy for mesangial proliferative glomerular nephritis, the effects of SMP-534 on TGF-β-induced ECM production in renal fibroblast cells, plus the...
effects of SMP-534 on a rat Thy-1 nephritis model, where anti-TGF-β antisera is effective (6), were investigated. In addition, the effect of SMP-534 on TGF-β signal transduction was examined.

**MATERIALS AND METHODS**

**Chemicals.** SMP-534 (Fig. 1) and SB-431542 were synthesized in our laboratories. SMP-534, SB-431542, staurosporine (Calbiochem, San Diego, CA), and U-0126 (Promega, Madison, WI) were dissolved in DMSO.

**Cell culture and proteoglycan synthesis assays.** Proteoglycan synthesis was quantified as described previously (3). Normal rat kidney fibroblasts cells (NRK-49F) were obtained from American Type Culture Collection (Manassas, VA) and cultured in DMEM (Invitrogen, Carlsbad, CA) containing 10% FBS. We aliquoted 2.5 × 10⁴ cells·100 μl⁻¹·well⁻¹ into a 96-well plate. The medium in the wells was changed the following day to DMEM containing 3 ng/ml TGF-β (Wako Pure Chemicals, Osaka, Japan), 18.5 kBq/well [³²P]ATP, and SMP-534. Supernatant was collected 24 h later and then subjected to SDS-PAGE in a conventional manner. After electrophoresis, the gel was dried with a gel drier, exposed to an imaging plate, and then analyzed by BAS2000 (Fuji Photo Film, Kanagawa, Japan).

**Collagen synthesis assay.** NRK-49F cells were cultured in DMEM containing 10% FBS. Cells in 12-well plates were made quiescent in DMEM containing 0.5% FBS for 24 h. After serum deprivation, cultures were incubated in DMEM containing 46.3 kBq/well [³²P]proline in the presence of TGF-β (5 ng/ml) and SMP-534. After 24-h incubation, the medium was harvested. Collagen synthesis during the 24-h treatment period was assessed by measuring [³²P]proline incorporation into pepsin-resistant, salt-precipitated extracellular/cell surface-associated proteins (30).

**ALK5 assay.** Kinase assays were performed with 4 μg/ml glutathione-S-transferase-ALK5 (Cascade Bioscience, Winchester, MA) and 1.4 μg/ml glutathione-S-transferase-Smad3 (Cascade Bioscience) in 50 mM HEPES, 5 mM MgCl₂, 1 mM CaCl₂, 1 mM dithiothreitol, and 3 μM ATP. Mixtures were incubated with 37 kBq [γ-³²P]ATP for 3 h at 30°C. Phosphorylated protein was captured on P-81 paper (Whatman, Maidstone, UK), washed with 75 mM phosphoric acid, and then counted by liquid scintillation.

**Western blot analysis of p38.** NRK-49F cells were cultured in DMEM containing 10% FBS. Cells in 10-cm dishes were made quiescent in DMEM without FBS. SMP-534 and staurosporine were added and incubated for a further 5 min. Cells were then washed and lysed. Western blot analysis was performed using anti-phosphorylated p38 antibody (Promega). Equal protein loading was verified using anti-p38 antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Detection of immunoreactive bands was carried out using an enhanced chemiluminescence system (Amersham Biosciences, Uppsala, Sweden).

**Western blot analysis of ERK.** Western blot analysis of ERK was performed in the same manner as for p38. NRK-49F cells were cultured and starved. Then SMP-534 and U-0126 were added. After a 60-min incubation, 1.5 ng/ml EGF (Wako Pure Chemicals) was added and incubated for a further 5 min. Cells were then washed and lysed. Western blot analysis was performed using anti-phosphorylated ERK1/2 and anti-ERK1/2 antibody (Santa Cruz Biotechnology).

**Quantitative TaqMan RT-PCR.** NRK-49F cells were cultured in DMEM containing 10% FBS. Quiescent cells in 10-cm dishes were treated by 3 ng/ml of TGF-β and 10 μM of SMP-534. After 24-h incubation, total RNA was extracted from cells with TRIzol reagent (Invitrogen). RT-PCR was performed on the ABI Prism 7700 sequence detection system using random hexamers from the TaqMan reverse transcription reagents and RT reaction mix (Applied Biosystems, Foster City, CA) to reverse transcribe the RNA, and TaqMan Universal PCR master mix and TaqMan gene expression assays (fibronectin: Rn00569575-m1, type I collagen: Rn00801649-m1, GAPDH: Rn9999916-m1) (Applied Biosystems) were used for the PCR step. The fibronectin and type I collagen mRNA expressions were normalized to the GAPDH mRNA expression. The relative standard curve method (Applied Biosystems) was used to calculate the amplification differences among all samples for each primer set.

**Animals.** Male Wistar rats (Charles River, Tokyo, Japan) weighing between 90 and 110 g were used; animals had free access to standard laboratory diet (CRF-1; Oriental Yeast, Tokyo, Japan) and water. Animals were kept in a controlled temperature room (24°C ± 2°C/55 ± 10%), with an illumination cycle from 8:00 to 20:00. All procedures were approved by the Sumitomo Pharmaceutical Committee on Animal Research.

**Induction of Thy-1 nephritis.** Thy-1 nephritis was induced by intravenous injection of a monoclonal antibody raised against rat Thy-1.1 antigen (clone OX-7; Biosource International, Camarillo, CA) at a dose of 0.75 mg/kg body wt.

**Experimental design.** Anti-Thy-1 monoclonal antibody was administered through the tail vein. Animals were then grouped into a vehicle-treated group and an SMP-534-treated group so that body weight was approximately even in both groups (n = 9). Starting on the day of anti-Thy-1 monoclonal antibody administration, SMP-534, suspended in 0.5% methylcellulose (vehicle), was orally administered through the tail vein. Animals were then grouped into a vehicle-treated group in the same manner. After 7 days of treatment, the right kidney was removed and hydroxyproline content was measured; the left kidney was removed after perfusion with phosphate-buffered formalin. Kidney sections were stained with periodic acid-Schiff (PAS).

**Quantification of hydroxyproline.** Hydroxyproline was quantified as described earlier (31). The cortical region of the kidney was homogenized, and the suspension was dried, followed by addition of 4 N NaOH. The mixture was heated at 100°C for 15 min and then neutralized with 1.4 M citric acid and centrifuged, and the supernatant was collected. Chloramine T solution and Ehrlich solution were added to the supernatant, and then the mixture was reacted at 65°C for 15 min. Absorbance at 550 nm was measured, and the concentration of hydroxyproline was calculated from a standard curve for hydroxyproline (Sigma, St. Louis, MO). Total protein concentration in kidney extract was quantified using bicinchoninic acid protein assay kit (Pierce, Rockford, IL). Hydroxyproline contents were normalized using protein concentration in the kidney extract.

**Light microscopy.** For morphometric analysis, sections were stained with PAS. Expansion of the glomerular matrix was scored in four levels from 0 to 3 and determined quantitatively (6). The scores were as follows: 0 = normal glomeruli; 1 = matrix expansion occurred in up to 50% of a single glomerulus; 2 = matrix expansion...
occurred in 50–75% of a single glomerulus; and 3 = matrix expansion occurred in 75–100% of a single glomerulus. For a single kidney specimen, more than 40 glomeruli were selected, and the mean score (mesangial expansion index) was determined. The investigator scoring the section was blinded.

Statistical analysis. All data are presented as means ± SD. Differences between individual groups were analyzed by Student’s t-test, Wilcoxon’s test, Tukey-Kramer’s test, William’s test, or Shirley-William’s test. Statistical calculations were performed using SAS software (SAS Institute, Cary, NC). P < 0.05 was considered statistically significant.

RESULTS

Effect of SMP-534 on TGF-β-induced proteoglycan, collagen, and fibronectin synthesis in cultured fibroblast cells. First, we used a cell-based assay to investigate the in vitro effect of our chemically synthesized SMP-534 on TGF-β action. Proteoglycan synthesis was monitored by SDS-PAGE and autoradiography using NRK-49F cells cultured in the presence of [35S]sulfate as substrate. As previously reported (3), sulfated proteoglycan production was increased by TGF-β treatment compared with non-TGF-β-treated controls. The molecular weight of proteoglycan as well as the amount was increased by TGF-β treatment. SMP-534 treatment of cultured cells together with TGF-β reduced TGF-β-induced proteoglycan production in a concentration-dependent manner. However, it is interesting to note that SMP-534 did not affect basal expression of proteoglycan in non-TGF-β-treated cells. SMP-534 (1 μM) inhibited 3 ng/ml TGF-β-induced proteoglycan synthesis by ~70% (Fig. 2, A and B).

Fig. 2. Effect of SMP-534 on TGF-β-induced proteoglycan accumulation. NRK-49F cells were stimulated with TGF-β (3 ng/ml) for 24 h in the absence (−) or presence (+) of different concentrations of SMP-534. Proteoglycan accumulation was evaluated by SDS-PAGE (A) and quantified by the BAS2000 image analyzer (B). Data are means ± SD (n = 3). ##P < 0.01 vs. TGF-β (−) and SMP-534 (−) by Tukey-Kramer’s test. **P < 0.01 vs. TGF-β (+) and SMP-534 (−) by Tukey-Kramer’s test.

Fig. 3. Effect of SMP-534 on TGF-β-induced collagen accumulation. NRK-49F cells were stimulated with TGF-β (5 ng/ml) for 24 h in the absence or presence of 2 μM SMP-534. Pepsin-resistant, salt-precipitated extracellular/cell surface-associated collagen was quantified with a liquid-scintillation counter. Data are means ± SD (n = 3). ##P < 0.01 vs. TGF-β (−) and SMP-534 (−) by Tukey-Kramer’s test. **P < 0.01 vs. TGF-β (+) and SMP-534 (−) by Tukey-Kramer’s test.

Fig. 4. Effect of SMP-534 on TGF-β-induced fibronectin and collagen mRNA expression. NRK-49F cells were stimulated with TGF-β (3 ng/ml) for 24 h in the absence or presence of 10 μM SMP-534. Fibronectin (A) and type I collagen (B) mRNA expression was evaluated by real-time RT-PCR. Fibronectin and type I collagen mRNA were subsequently normalized to GAPDH mRNA. Levels of fibronectin and type I collagen mRNA were expressed as fold difference against vehicle-treated cells. Data are means ± SD (n = 5). ##P < 0.01, *P < 0.05 vs. TGF-β (−) and SMP-534 (−) by Tukey-Kramer’s test. **P < 0.01, *P < 0.05 vs. TGF-β (+) and SMP-534 (−) by Tukey-Kramer’s test.
Collagen synthesis was next investigated in a similar fashion where pepsin-resistant salt-precipitated protein was measured after NRK-49F cells were cultured using $[3H]$proline (30). As shown in Fig. 3, TGF-β increased collagen production by 1.5-fold, and 2 μM SMP-534 reversed this effect. Again SMP-534 did not affect spontaneous collagen synthesis in this cell line.

To ascertain whether SMP-534 affects TGF-β-induced ECM synthesis at transcriptional levels, ECM-related mRNA expression was monitored by real-time RT-PCR using NRK-49F cells. As previously reported (16), TGF-β increased fibronectin and type I collagen mRNA expression by 1.6-fold and 1.2-fold, respectively. A dose of 10 μM SMP-534 significantly reduced TGF-β-induced mRNA expression (Fig. 4). Consistent with the results of the proteoglycan and collagen assay, SMP-534 did not affect spontaneous mRNA expression of these genes.

No cell toxicity was observed from addition of SMP-534 up to 30 μM (data not shown).

**Effect of SMP-534 on rat Thy-1 nephritis model.** To evaluate the antifibrotic activity of SMP-534 in vivo, we examined the effect of SMP-534 on rat Thy-1 nephritis models. Thy-1 models are well-known animal models for kidney fibrosis, and histological analysis has demonstrated that antisera against TGF-β prevents the accumulation of ECM in kidney (6). In the present study, after injection of anti-Thy-1 antibody, oral administration of SMP-534 was performed daily for 1 wk. The dose of SMP-534 ranged from 1.5 to 50 mg/kg. As shown in Fig. 5A, no significant change in body weight was observed from SMP-534 treatment.

In PAS-stained sections of kidney, marked accumulation of ECM was observed in the Thy-1 nephritis group compared with the control group. SMP-534 treatment clearly ameliorated this pathological change. As shown in Fig. 5B, mesangial expansion index was significantly lowered in SMP-534-treated group compared with vehicle-treated group. Representative light micrographs of glomeruli were shown in Fig. 5C.

In accordance with this finding, hydroxyproline content in the cortex region of the kidney was significantly increased in the anti-Thy-1 antibody-treated group compared with the normal control group [11.3 (SD 0.7) vs. 16.1 (SD 1.6) μg/mg protein; $n = 9$, $P < 0.01$]. SMP-534 dose dependently lowered...
hydroxyproline content, with this effect statistically significant at more than 5 mg/kg (Fig. 5D). These results clearly demonstrate that SMP-534 inhibits the accumulation of extracellular matrix in in vivo as well as in vitro cell culture systems.

**Effect of SMP-534 on ALK5 kinase activity.** Findings of the inhibitory actions of SMP-534 in both in vitro and in vivo studies prompted us to evaluate the action mechanism of SMP-534 underlining modulation of TGF-β activity. Although SMP-534 is a rather smaller molecule than TGF-β protein, we investigated whether SMP-534 showed sufficient antagonistic action to TGF-β receptor to block TGF-β binding. In the receptor binding experiment using 125I-labeled TGF-β, SMP-534 did not work as an antagonist of TGF-β type II receptor (data not shown). This indicates the possibility that a small molecule such as SMP-534 might affect receptor kinase activity instead of blocking the binding of TGF-β. In fact, SB-431542, a small molecule inhibitor of TGF-β type I receptor kinase, has recently been reported (9) and shown to inhibit the p38 activation induced by 0.1 ng/ml TGF-β with 40%.

**Effect of SMP-534 on ALK5 kinase activity.** Findings of the inhibitory actions of SMP-534 in both in vitro and in vivo studies prompted us to evaluate the action mechanism of SMP-534 underlining modulation of TGF-β activity. Although SMP-534 is a rather smaller molecule than TGF-β protein, we investigated whether SMP-534 showed sufficient antagonistic action to TGF-β receptor to block TGF-β binding. In the receptor binding experiment using 125I-labeled TGF-β, SMP-534 did not work as an antagonist of TGF-β type II receptor (data not shown). This indicates the possibility that a small molecule such as SMP-534 might affect receptor kinase activity instead of blocking the binding of TGF-β. In fact, SB-431542, a small molecule inhibitor of TGF-β type I receptor kinase, has recently been reported (9) and shown to inhibit the in vitro Smad3 phosphorylation and ECM production (20). To compare the mechanisms of action at the most upstream step of TGF-β signaling, the effect of SMP-534 on ALK5 activity was examined in a cell-free assay (Fig. 6). As previously described, Smad3 was phosphorylated by addition of constitutively active ALK5, and SB-431542, an inhibitor of ALK5, inhibited kinase activity (9). Under these conditions, SMP-534 did not inhibit ALK5 kinase activity up to 10 μM.

**Effect of SMP-534 on p38 activation.** Because SMP-534 inhibited TGF-β-induced ECM synthesis without inhibiting ALK5 kinase, the effect of SMP-534 may be related to intracellular events of TGF-β signaling. It has been reported that p38 is one of the major signaling molecules involved in TGF-β signal transduction. Therefore, the effect of SMP-534 on activation of p38 in response to TGF-β stimulation was examined using a Western blot assay. As previously reported (15), p38 was phosphorylated after 30-min treatment of NRK-49F cells with TGF-β. In this study, staurosporine, a nonspecific protein kinase inhibitor, inhibited TGF-β-induced p38 activation. Similarly, activation was clearly blocked by treatment with SMP-534 (Fig. 7). SMP-534 at 10 μM inhibited 0.1 ng/ml TGF-β-induced p38 activation by ~40%.

**Effect of SMP-534 on ERK activation.** SMP-534 inhibited TGF-β-induced ECM synthesis by inhibiting p38 activation. It is important to determine how specifically SMP-534 affects MAPK family activation by other corresponding growth factors. It is known that EGF activates MAP kinases ERK1 and ERK2 through a signal transduction pathway, which is mediated by a series of kinases, including EGF receptor kinase, the MAPKKK, Raf, and MEK1 and MEK2 (5). The effect of SMP-534 on EGF-induced ERK1 and ERK2 activation in NRK-49F cells was examined by Western blot assay. ERK1 and ERK2 are rapidly phosphorylated after 5-min treatment with EGF. This induction was not affected by treatment with SMP-534.
SMP-534 up to 100 μM (Fig. 8). In contrast, EGF-induced ERK1 and ERK2 activation was completely inhibited by addition of MEK1/2 inhibitor, U-0126 (13). Similarly, SMP-534 had no effect on PDGF-induced ERK1 and ERK2 activation in NRK-49F cells (data not shown). As far as we could determine, SMP-534 inhibits MAPK with some specificity to TGF-β signaling.

DISCUSSION

In recent years, the number of patients with end-stage renal disease (ESRD) has dramatically increased worldwide. Prevention of ESRD is a major challenge facing us today. The therapeutic success of both angiotensin-converting enzyme inhibitors (ACEIs) (23) and angiotensin AT1-receptor antagonists (ARBs) (8) in renal diseases has demonstrated that ANG II is a central effector in renal diseases. It is thought that prevention of progression to ESRD by ACEIs and ARBs derives from their renal protection effect, independent of blood pressure control. However, the renal protection effects of both drugs are insufficient to prevent the progression of ESRD completely as all clinical trials conducted so far have demonstrated that inhibition of ANG II production with ACEIs or ARBs only slows progression to ESRD (34). Therefore, additional intervention including treatment with novel therapeutic agents is needed in combat chronic renal disease.

Considerable experimental evidence supports a key role for TGF-β in the pathogenesis of chronic renal disease, which is mainly characterized by the accumulation of ECM (7). TGF-β is one of the most potent fibrotic cytokines released and has been implicated in a variety of human fibrotic diseases, including glomerulonephritis (33), diabetic nephropathy (32), liver cirrhosis (22), and lung fibrosis (2). Because TGF-β is a potent stimulus for ECM synthesis, inhibition of the TGF-β signal may be beneficial in fibrotic disorders. There is therefore intense interest in the development of therapeutic use of specific small molecule inhibitors of ECM production induced by TGF-β. Screening of our internal compound collection and optimization of the screening hits identified SMP-534 as an inhibitor of ECM production induced by TGF-β.

As reported by Lapin’s group (17, 20), SB-431542 prevented TGF-β-induced elevation of fibronectin, plasminogen activator inhibitor 1, and collagen mRNA. They demonstrated that SB-431542 was a potent inhibitor of ALK5 kinase with >100-fold selectivity against p38 MAPK and 25 other kinases. To compare the mechanism of action of SMP-534 with SB-431542, the effect on ALK5 Ser/Thr kinase activity was investigated and found that SMP-534 did not inhibit ALK5 kinase activity. In addition, SMP-534 did not affect TGF-β binding to TGF-β type-II receptor (data not shown). Therefore, it was assumed that SMP-534 inhibits TGF-β signal transduction downstream from ALK5. To ascertain the action mechanism of how SMP-534 inhibits TGF-β-induced ECM production, the effect of SMP-534 on p38 activation, which has important roles in TGF-β signaling, was examined. SMP-534 inhibited TGF-β-induced p38 activation (phosphorylation), indicating that inhibition of p38 signaling is involved in the action mechanism of SMP-534, at least in part.

Because SMP-534 may have potentiating effects on a range of kinases and other effector molecules, it is important to investigate the specificity of SMP-534. Representing growth factor signals, the effect of SMP-534 on EGF-induced ERK activation was investigated. As shown in Fig. 8, SMP-534 had no effect on EGF-induced ERK activation at concentrations as high as 100 μM. In addition, we have observed that SMP-534 did not affect 22 of the kinases studied, including PKC, PKB (Akt), and Src (data not shown). Although the effect of SMP-534 on several other possible signaling pathways involving TGF-β action should be examined, our data suggest that SMP-534 selectively inhibits p38 activation induced by TGF-β. We have demonstrated here that SMP-534 inhibited ECM accumulation only when TGF-β was added to the cell and did not affect basal ECM production. To evaluate the antifibrosis activity of SMP-534, we used a rat Thy-1 nephritis model, which is characterized by ECM accumulation in kidney and is often used in examination of antifibrotic agents, including anti-TGF-β antiserum (6). One week after intravenous injection of antibody against Thy-1 molecule, an increase of PAS-stained ECM accumulation in the glomeruli was histologically observed. Consistent with this finding, hydroxyproline content, which is known to reflect collagen content, was significantly increased in the cortical region of the kidney. Oral administration of SMP-534 dose dependently prevented histological alternations and lowered hydroxyproline content, demonstrating that SMP-534 is effective in this model, as assessed both biochemically and histologically. As far as we could determine, however, renal function was not damaged in this model. Therefore, it is still unclear whether SMP-534 has a renoprotective action. To examine whether the antifibrotic activity of SMP-534 has a favorable effect on renal function, more detailed studies are necessary using other animal models such as diabetic db/db mice (19, 35) and 5/6 nephrectomized rats (1).

Pirfenidone is a recently reported antifibrotic agent that inhibits TGF-β-induced collagen production at 100 to ~1,000 μM (11) and prevents ECM accumulation in rat Thy-1 nephritis at 500 mg/kg (27). In addition to kidney fibrosis models, efficacies of pirfenidone are reported in several animal models, including lung (18), liver (14), and cardiac fibrosis (12). Therefore, it is interesting to examine whether SMP-534 shows efficacy in such fibrosis models.

In summary, SMP-534 prevented TGF-β-induced ECM production in fibroblast cells by inhibiting intracellular TGF-β signaling. Oral administration of SMP-534 improved glomerulonephritis in a rat Thy-1 nephritis model. Because TGF-β is implicated in a variety of other fibrotic diseases in addition to kidney fibrosis, SMP-534 may have great potential for improving a variety of fibrotic diseases, including liver cirrhosis, skin fibrosis, and lung fibrosis.

REFERENCES


