Inhibition of spleen tyrosine kinase (Syk) suppresses renal fibrosis through anti-inflammatory effects and down regulation of the MAPK-p38 pathway

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1. Introduction

Renal fibrosis, characterized by tubular atrophy, interstitial inflammation, and excessive accumulation of extracellular matrix (ECM) proteins, is the final common pathway resulting in progressive loss of renal function in various types of chronic kidney disease (CKD) (Jung et al., 2012). Unilateral ureteral obstruction (UUO) is a representative experimental animal model widely used to investigate the pathophysiology of renal fibrosis (Chen et al., 2014). This model reflects the inflammatory responses and fibrosis observed in human CKD. UUO is characterized by renal tubular dilatation, interstitial inflammation, macrophage and lymphocyte infiltration, myofibroblast activation, and interstitial ECM accumulation (Eddy, 2014). Infiltrated inflammatory cells not only generate numerous chemokines, but also release profibrotic cytokines and growth factors, including transforming growth factor β1 (TGF-β1), which then act on renal tubular cells and resident fibroblasts to promote renal fibrosis (Liu, 2011). Chronic interstitial inflammation, followed by functional loss of renal parenchyma and interstitial fibrosis, result in the impairment of renal function (Klahr and Morrissey, 2002). Therefore, suppressing the inflammatory response could attenuate progressive renal fibrosis.

Spleen tyrosine kinase (Syk), a 72 kDa protein, is a member of the Src family of non-receptor tyrosine kinases. Syk is highly expressed in hematopoietic cells, where it has a critical function in classical immune receptor signaling. Syk has a well-characterized role in the intracellular signal transduction pathway associated with the B-cell surface receptor (Geahlen, 2009) and the activating Fc...
receptors expressed on various immune effector cells, including myeloid cells (Kiefer et al., 1998) and mast cells (de Castro, 2011). Therefore, Syk has emerged as a potential therapeutic target in hematologic malignancy, allergy and autoimmunity. Syk activation has also recently been demonstrated in vascular smooth muscle cells after angiotensin II stimulation (Mugabe et al., 2010), affecting osteoclast and synoviocyte function (Mun et al., 2009; Tohyama and Yamamura, 2009). The down-stream effectors of Syk signaling pathways include protein kinase C (PKC) (Chang et al., 2012), protein tyrosine kinase 2 (PYK2)-mediated cytoskeletal rearrangement (Suen et al., 1999), AKT (Chen et al., 2011), MAPKs (Yoshida et al., 2011), and Nlrp3 inflammasome pathways (Chang et al., 2012; Gross et al., 2009). Recent studies have identified a number of new Syk functions beyond the immune system. Syk activation has been demonstrated in both animal models and humans with proliferative glomerulonephritis (McAdoo et al., 2015; Ryan et al., 2011; Smith et al., 2010). However, the involvement of Syk in the modulation of the fibrogenic properties of TGF-β1 in renal interstitial fibrosis and tubulointerstitial inflammation has not yet been investigated. In the present study, we evaluated the effects of Syk pathway inhibition on renal fibrogenesis in vivo and in vitro.

2. Materials and methods

2.1. Ethics statement

Animal experiments were carried out in accordance with the guidelines issued by the Animal Care and Ethics Committee on Research at Chang Gung Memorial Hospital. This study was approved by the Animal Care and Use Committee of Chang Gung Memorial Hospital [Approval number 2011121908].

2.2. Antibodies and reagents

Bovine calf serum (BCS) and Dulbecco’s modified Eagle’s medium (DMEM), were purchased from HyClone Laboratories, Inc. (South Logan, UT, USA). A Syk inhibitor (2-[(3,4-dimethoxyphenyl)-imidazo[1,2-b]pyrimidin-5-ylamino]-nicotinamide dihydrochloride, BAY 61-3606) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). α-smooth muscle actin (α-SMA) antibody was obtained from Sigma Chemical Co. Phospho-specific Syk, Smad2, ERK1/2, JNK, p38, TAK1, and MKK3/6 antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Collagen I and IV antibodies were obtained from Southern Biotech (Birmingham, AL, USA). Fibronectin and β-actin antibodies were purchased from Abcam (Cambridge, MA, USA). All other reagents were obtained from Sigma Chemical Co. Recombinant human TGF-β1 was purchased from R&D Systems (Minneapolis, MN, USA).

2.3. Mouse models of kidney fibrosis

Unilateral ureteral obstruction (UUO) was induced in adult male B6 mice (n = 6; 3–5 months old, 20–25 g) under 2.5% avertin-induced anesthesia. The left kidney and ureter were exposed via a flank incision, following which the ureter was ligated at two points proximal to the kidney with 6–0 silk. The wound was closed in layers. In sham animals (n = 6), the kidney and ureter were exposed, but the ureter was not tied. Mice with UUO were intraperitoneally administered the Syk inhibitor (20, 40 mg/kg body weight per day) or saline 1 day before the UUO surgery and daily thereafter. Both the obstructed and contralateral kidneys were harvested 7 days after surgery.

2.4. Kidney tissue preparation

Mice were anesthetized and sacrificed, and both kidneys were then harvested. Kidneys were hemi-sectioned and portions were snap frozen in liquid nitrogen for real-time-PCR or western blot analysis. Some kidneys were fixed in 10% neutral buffered formalin at 4 °C for 12 h, processed, embedded in paraffin wax, sliced into 4 mm sections, and stored at room temperature until use.

2.5. Renal fibrosis analysis

To assess renal fibrosis, Gomori’s trichrome staining was performed according to the manufacturer's instructions (Leica Biosystems Richmond, Inc., Richmond, IL, USA). Twenty individual high-power fields (magnification, 100×) per kidney were analyzed. The percentage area occupied by collagen tissue (blue color) was analyzed using computer-assisted image analysis software (Meta-Morph, version 4.6, Universal Imaging Corporation, Downingtown, PA, USA).

2.6. Immunofluorescence and immunohistochemical staining of F4/80 to assess macrophage infiltration

Paraffin sections (4 μm thick) of renal tissue were analyzed by immunohistochemistry using monoclonal antibodies against F4/80 (1:50). To detect F4/80, a biotinylated secondary antibody (1:300, incubated for 30 min at room temperature) and streptavidin–biotin–peroxidase (incubated for 30 min at room temperature) were used. Sections were counterstained with hematoxylin, dehydrated and mounted. For each kidney, twenty individual high-power fields (magnification, 200×) per kidney were analyzed and representative images are presented.

2.7. Quantitative real-time PCR analysis

Real-time-PCR was performed using total RNA isolated from the kidney samples in an ABI-Prism 7000 with SYBR Green I as a double-stranded DNA-specific dye, according to the manufacturer’s instructions (PE-Applied Biosystems, Cheshire, UK). Expression of 18S mRNA was used as an internal control. Primer sequences are listed in Table 1. Primers were constructed to be compatible with a single RT-PCR thermal profile (95 °C for 10 min, and 40 cycles of 95 °C for 30 s, and 60 °C for 1 min). The change in gene expression was determined for the sham, UUO, and UUO + Syk inhibitor treated mice.

2.8. Cell culture and experimental treatments

NRK-49F rat fibroblasts (ATCC, Manassas, VA, USA) were grown in DMEM containing 5% BCS at 37 °C in an atmosphere containing 5% CO2. Cells were seeded and allowed to adhere overnight, following which the medium was changed to DMEM containing 0.1% FBS. Cells were stimulated with recombinant human TGF-β1 (1 ng/ml) for the indicated periods before harvesting. Alternatively, NRK-49F cells were pre-incubated with the Syk inhibitor for 30 min before TGF-β1 treatment. Total RNA was extracted for real-time PCR, and total cell lysates were extracted for western blot analysis. All measurements were performed at least in triplicate.

2.9. Western blot analysis

Total cellular protein was extracted as previously described. Equal amounts of proteins were mixed with an equal volume of reducing SDS sample buffer and boiled at 95 °C for 5 min. Protein samples were resolved by 10% SDS-PAGE and electroblotted on
Table 1
Forward and reverse primers used for quantitative real-time reverse transcriptase PCR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Type</th>
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<tr>
<td>Collagen I</td>
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<td>Forward</td>
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<td>Reverse</td>
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<tr>
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<tr>
<td>TGF-β1</td>
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<td></td>
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<td>CTGF</td>
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<tr>
<td>18S rRNA</td>
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Abbreviations: Collagen I, collagen type-I; α-SMA: α-smooth muscle actin; TGF-β1, transforming growth factor-β1; CTFG, connective tissue growth factor; MRC, macrophage mannose receptor; TLR2, toll-like receptor 2; TLR4, toll-like receptor 4.

nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). After electrophotming, nonspecific binding was blocked with 5% nonfat milk. The membrane was incubated with primary antibodies overnight at 4°C and incubated with horseradish peroxidase–conjugated secondary antibodies for 1 h at room temperature. Primary antibodies against the following proteins were used at 1:1000 dilutions unless otherwise indicated: collagen I, collagen IV, α-SMA, phospho-Smad2, phospho-Smad3, phospho-ERK1/2, phospho-p38, phospho-JNK, anti-tubulin (1:20,000), and anti-β-actin (1:20,000). Immunoreactive bands were visualized using enhanced chemiluminescence (Amersham, Arlington Heights, IL, USA) as previously described.

2.10. Statistical analysis

All experiments were conducted at least three times. Data depicted in graphs represent the mean ± S.E. for each group. One-way analysis of variance (ANOVA) was conducted for multiplegroup comparisons, and Bonferroni’s post-hoc analysis was used to evaluate the significance of paired groups in the animal study. In the study, Student’s t-test was used to calculate the significance of the difference in the results obtained between different conditions. In all analyses, P values less than 0.05 were considered statistically significant.

3. Results

3.1. Administration of a Syk inhibitor inhibited kidney fibrosis in UUO mice in a dose-dependent manner

No morphological or molecular differences were observed in the non-obstructed contralateral kidneys of mice from either of the experimental groups (UUO+ saline and UUO+ Syk inhibitor; data not shown). Compared with the sham control kidneys (Fig. 1A, upper left panel), kidneys from mice with UUO developed a severe tubulointerstitial injury consisting of marked tubular dilatation and atrophy, interstitial inflammation, and fibrosis (Fig. 1A, upper right panel). Glomeruli and vessels were well preserved. Masson’s trichrome staining revealed intense deposition of ECM in the tubulointerstitium of obstructed kidneys. Administration of the Syk inhibitor attenuated ECM deposition in a dose-dependent manner (Fig. 1A, lower panels). Renal fibrosis analysis showed that the Syk inhibitor attenuated UUO-induced tubulointerstitial fibrosis in a dose-dependent manner compared with that in the UUO+ saline group (Fig. 1B). The effects of the Syk inhibitor on mRNA expression of genes encoding α-SMA, type I collagen, fibronectin, and TGF-β1 were analyzed in kidney tissue lysates. RT-PCR analysis revealed that UUO injury significantly increased the expression of genes encoding α-SMA, type I collagen, fibronectin, and TGF-β1 and CTGF, whereas treatment with the Syk inhibitor attenuated this increased expression (Fig. 2A–E). In agreement with the mRNA expression results, western blotting showed that Syk inhibition had a similar inhibitory effect on protein levels (Fig. 2F). Protein expression results for α-SMA, collagen I, and fibronectin are shown in Fig. 2G–I. Taken together, these data showed that Syk inhibitor treatment significantly attenuated tubulointerstitial fibrosis in UUO mice.

3.2. Syk inhibition suppressed the tubulointerstitial inflammation in UUO mice

To determine whether tubulointerstitial inflammation is suppressed by Syk inhibition, F4/80 macrophage immuno-staining was performed. Faint staining was observed in sham-operated kidneys. However, F4/80 expression was significantly increased in the tubulointerstitial area of saline-treated UUO mice, and Syk inhibition decreased expression in UUO mice in a dose-dependent manner (Fig. 3A). In addition, mRNA expression of Toll-like receptor 2 (TLR2) and TLR4 was increased in UUO mice and suppressed by Syk inhibition (Fig. 3B). mRNA expression of arginase-1 (M2 macrophage marker) was increased in UUO mice and inhibited by Syk inhibition (Fig. 3B). Furthermore, mRNA expression of inflammasome (ASC, caspase-1, Nlrp3, IL-1β) was increased in UUO mice, and Syk inhibition suppressed inflammasome activation in a dose-dependent manner (Fig. 3C).

3.3. TGF-β1 activation of fibroblast-myofibroblast transformation is inhibited by Syk inhibition

To evaluate the intracellular signaling pathways involved in the anti-fibrotic effects of Syk inhibition, fibroblast-myofibroblast transformation of the NRK-49F rat renal fibroblast cell line, was induced by TGF-β1 treatment (1 ng/mL). Phospho-Syk (p-Syk) expression was increased by TGF-β1 stimulation in NRK-49F cells, and pre-treatment with the Syk inhibitor (1.0 μM) before TGF-β1 treatment suppressed p-Syk expression (Fig. 4A). Further, production of type I and type IV collagens, as well as α-SMA was significantly increased in NRK-49F cells in response to TGF-β1 stimulation. Pre-treatment of NRK-49F cells with the Syk inhibitor for 30 min resulted in dose-dependent inhibition of α-SMA, type I collagen, and fibronectin expression in NRK-49F cell lysates after TGF-β1 stimulation (Fig. 4B–F). These results demonstrate that Syk inhibition suppresses TGF-1 induced fibroblast-myofibroblast transformation in NRK-49F cells.

3.4. Effects of Syk inhibition on the TGF-β1 signaling pathway were associated with down-regulation of p38

The effects of Syk inhibition on the TGF-β1 signaling pathway were further evaluated. Both the classical TGF-β1/Smad pathway and non-Smad pathways were investigated. Western blot analysis demonstrated that treatment with the Syk inhibitor did not affect TGF-β1-stimulated phosphorylation of Smad2 in the canonical TGF-β1/Smad pathway in NRK-49F cells (Fig. 5A). However, p38...
activation in NRK-49F cells treated with TGF-β1 was suppressed in a time-dependent by Syk inhibition, whereas activation of the ERK1/2 and JNK pathways was not affected (Fig. 5B). Similar to the observation in NRK-49F cells in vitro, protein expression of p-Syk, p-ERK1/2 and p-p38 was increased in obstructed kidneys compared with that observed in sham kidneys. Expression of p-Syk and p-p38, but not p-ERK1/2, decreased in a dose-dependent manner in UUO kidneys treated with the Syk inhibitor (Fig. 5C).

3.5. Syk inhibition suppressed upstream kinases of p38 after TGF-β1 stimulation

Because only p38 activation by TGF-β1 was suppressed by Syk inhibition, we further investigated whether the upstream kinases of p38 were influenced by Syk inhibition. TAK1 and MKK 3/6 are important upstream kinases of the p38 signaling pathway after TGF-β1 stimulation. Using western blot analysis, we demonstrated that the expression of p-TAK and p-MKK after TGF-β1 stimulation in MRK-49F cells was inhibited by Syk inhibition (Fig. 6).

4. Discussion

Tubulointerstitial renal fibrosis is the final common pathway of various chronic renal diseases. Activation of myofibroblasts by TGF-β1 and tubulointerstitial inflammation leads to ECM production, followed by progressive fibrosis and tubular atrophy. In previous studies, Syk was been shown to be an important therapeutic target for antibody-mediated glomerulonephritis (Kim et al., 2012; McAdoo et al., 2015; Ryan et al., 2011; Smith et al., 2010). However, the role of Syk in tubulointerstitial fibrosis has not been characterized. We showed that Syk inhibitor treatment attenuated tubulointerstitial fibrosis in the obstructed kidneys in UUO model mice. In addition, we demonstrated that the inhibitory effect of Syk inhibitor treatment on TGF-β1-induced fibroblast-myofibroblast transformation was partly mediated through down-regulation of p38 activation by TGF-β1.

Seven days after ureteral ligation, obstructed kidneys displayed typical features of the UUO model. Our study demonstrated that tubulointerstitial fibrosis was significantly reduced in the UO kidney in Syk inhibitor-treated mice compared with that in saline-treated mice. Moreover, a marker of myofibroblast activation, α-SMA, and expression of other fibrotic markers such as, collagen I and fibronectin in UO kidney were all attenuated in a dose-dependent manner in Syk inhibitor-treated UO mice compared with those in saline-treated UO mice. Because the severity of fibrosis is related to the extent of tubulointerstitial inflammation in the UUO model, the anti-inflammatory effects of Syk inhibition were further investigated.

During the development of fibrosis, inflammation is often a prelude to fibrosis. Both innate immunity and adaptive immunity are involved in the initiation and regulation of the fibrotic process. Macrophages have been suggested to play an important role in the pathogenesis of UUO-induced renal fibrosis, by secreting pro-inflammatory cytokines and inducing the death of tubular cells, leading to tissue injury and the development of renal fibrosis (Shen et al., 2014; Sung et al., 2007). Macrophages can be activated via a number of intracellular signaling pathways that induce production renal injury mediators. Thus, targeting secreted macrophage signaling pathways is a potential therapeutic strategy to suppress macrophage-mediated renal injury. Syk activation has been demonstrated in macrophages under LPS or minimally oxidized low-density lipoprotein (mMDD) stimulation, and it leads to the induction of inflammatory cytokines (Miller et al., 2012). In our study, F4/80 staining for macrophages showed that the infiltration was significantly reduced in Syk inhibitor-treated UO mice. TLR signaling is involved in renal fibrogenesis (Campbell et al., 2011; Skugninna et al., 2011). TLR2 and TLR4 expression is increased in UO mice, and TLR2- and TLR4- knockout mice have been shown to display improved renal function and decreased fibrosis after UUO (Braga et al., 2012). In addition, activation of M2 macrophages and the stimulation of a TH2 immune response have been demonstrated in a MyD88-dependent manner in UUO mice (Braga et al., 2012). We showed that Syk inhibitor treated UO mice display significantly reduced expression of genes encoding TLR2 and TLR4 compared with that observed in saline-treated UO mice. mRNA expression of an M2 macrophage marker (arginase-1) was significantly reduced in Syk inhibitor treated UUO mice.

We also showed that Nlrp3 inflammasome activation in UO mice is also inhibited by treatment with the Syk inhibitor. Syk is essential for the production of the Nlrp3 inflammasome required...
Fig. 2. Syk inhibitor (Syki) reduces expression of α-smooth muscle actin (α-SMA), collagen I, and fibronectin in UUO kidneys. A unilateral ureteral obstruction (UUO) model was established using adult male B6 mice. Sham animals had their kidneys exposed but their ureters were not tied. Mice with UUO were intraperitoneally administered a Syk inhibitor (20, 40 mg kg
−1day) or saline 1 day before UUO surgery and daily thereafter. Kidneys were harvested 7 days after surgery. qPCR analysis of the mRNA expression of genes encoding α-SMA (A), collagen I (B), fibronectin (C), TGF-β1 (D), and CTGF (E), in sham, UUO, and UUO + Syki kidneys. Data are expressed relative to expression in sham-operated kidneys. Kidney tissue lysates were also subjected to immunoblot analysis using specific antibodies against α-SMA, collagen I, fibronectin, and β-actin (F). Protein expression levels of α-SMA (G), collagen I (H), and fibronectin (I) were analyzed using western blotting, quantified by densitometry, and normalized to β-actin levels. Each bar represents the mean ± S.E. (n = 6 in each group). *P < 0.05 between the sham and UUO group; # P < 0.05 between the UUO and UUO + Syki group.
Fig. 3. Syk inhibitor (Syki) reduces macrophage infiltration and inflammasome expression in UUO kidneys.

A unilateral ureteral obstruction (UUO) model was established using adult male B6 mice. Sham animals had their kidneys exposed but their ureters were not tied. Mice with UUO were intraperitoneally administered a Syk inhibitor (20, 40 mg kg⁻¹ day) or saline 1 day before UUO surgery and daily thereafter. Kidneys were harvested 7 days after surgery.

(A) Photomicrographs illustrating F4/80 macrophage staining of kidney tissue from mice in various treatment groups. (B) The mRNA expression in kidney tissues of genes encoding Toll-like receptor 2 (TLR2), TLR4, M1 macrophage markers (MRC, IL-23), and an M2 macrophage marker (arginase-1). (C) mRNA expression of genes encoding factors associated with the inflammasome (ASC, caspase-1, Nlrp3, IL-1β) in kidney. Data are expressed relative to expression in sham-operated kidneys. Each bar represents the mean ± S.E. (n = 6 in each group). *P < 0.05 between the sham and UUO group; # P < 0.05 between the UUO and UUO + Syki group.
Fig. 4. Syk inhibitor (Syki) inhibits transforming growth factor-β1 (TGF-β1)-induced fibroblast-myofibroblast transformation. (A) Cultured NRK-49F cells were incubated with TGF-β1 (1 ng/mL) for 15–120 min in the presence or absence of Syki (1 μM). Cell lysates were subjected to immunoblot analysis using antibodies against phospho-Syk and β-actin. (B) NRK-49F cells were pre-incubated in the presence or absence of Syki (0.1–1 μM) for 30 min. Next, cells were stimulated with TGF-β1 (1 ng/mL) for 24 h before harvesting. The cell lysate was also subjected to immunoblot analysis using antibodies against α-SMA, collagen I, collagen IV, fibronectin, and β-actin. Representative immunoblots from three independent experiments are shown. Protein expression levels of α-SMA (C), collagen I (D), collagen IV (E), and fibronectin (F) were analyzed using western blotting, quantified by densitometry, and normalized to β-actin levels. Each bar represents the mean ± S.E. of three independent experiments. *P < 0.05 between the control and TGF-β1 treatment; # P < 0.05 between the TGF-β1 treatment and TGF-β1 + Syki groups. C: control, TGF: TGF-β1.
Fig. 5. Effects of Syk inhibitor (Syki) in the non-Smad TGF-β pathway were associated with down-regulation of p38. Cultured NRK-49F cells were incubated with TGF-β1 (1 ng/mL) for 15–120 min in the presence or absence of the Syk inhibitor (1 μM). (A) Cell lysates were subjected to immunoblot analysis using antibodies against phospho-Smad2 (P-Smad2) and tubulin. (B) Cell lysates were subjected to immunoblot analysis using antibodies against phospho-ERK1/2 (P-ERK1/2), phospho-p38 (P-p38), phospho-JNK (P-JNK), and tubulin. Representative immunoblots from three experiments are shown. Protein expression levels of P-p38 were analyzed using western blotting, quantified by densitometry, and normalized to tubulin levels. Each bar represents the mean ± S.E. of three independent experiments. *P<0.05 versus the corresponding group (control or TGF-β1) at the same time duration after TGF-β1 treatment; C: control, T: TGF-β1; (C) Kidney tissue lysates were subjected to immunoblot analysis using specific antibodies against phospho-Syk (P-Syk), phospho-ERK1/2 (P-ERK1/2), total ERK1/2 (T-ERK1/2), phosphor-p38 (P-p38), and β-actin. Representative immunoblots from three independent experiments are shown.
for antifungal immunity (Gross et al., 2009; Poeck and Ruland, 2010). In monosodium urate (MSU)-mediated activation of monocytes and neutrophils, Syk phosphorylation was shown to be required for subsequent Nlrp3 inflammasome activation and IL-1β production (Desaulniers et al., 2001; Martinon et al., 2009). Activation of Nlrp3 inflammasome has also been demonstrated to promote inflammation and fibrosis in the UUO model (Vilaysane et al., 2010; Wang et al., 2015). Our study showed that Syk inhibition also suppresses Nlrp3 inflammasome expression in UUO mice. Therefore, the effect of Syk inhibition on tubulointerstitial fibrosis is partially mediated through innate immunity via a reduction in macrophage infiltration and macrophage-mediated renal inflammation.

We further used NRK-49F cells as an in vitro model to investigate the mechanism by which Syk inhibition affects signaling pathways. In NRK-49F cells, the Syk inhibitor inhibited production of collagen I, collagen IV, and α-SMA in a dose-dependent manner in TGF-β1-stimulated renal fibroblast-myofibroblast transformation. TGF-β1 associated downstream signaling pathways potentially affected by Syk inhibition were further investigated. Syk inhibition did not affect Smad2 phosphorylation in canonical TGF-β/Smad pathways. However, MAPK signaling pathways (ERK1/2, p38, and JNK) are also important pathways downstream of TGF-β1 signaling. We showed that p38 activation by TGF-β1 was suppressed in Syk inhibitor treated NRK-49F cells, whereas ERK1/2 and JNK activation were not affected. More importantly, p-p38 activation in UUO kidney protein extracts was significantly suppressed in UUO + Syk inhibitor mice compared with that in saline treated UUO mice. These results indicate that Syk inhibitor mediated suppression of TGF-β1 induced myofibroblast activation is associated with p38 inhibition.

The relationship between Syk and p38 has been evaluated in previous studies. In B-cells stimulated by oxidative stress, Syk was required for p38 activation and the regulation of cell-cycle progression (He et al., 2002). Ryan et al. (2011) demonstrated that in a model of rat nephrotoxic serum nephritis (NTN), Syk inhibitor treatment reduced the acute glomerular neutrophil influx and
pro-inflammatory response. The protective effects observed were associated with a reduction in glomerular JNK and p38 activation (Ryan et al., 2011). However, whether the upstream kinases of p38 are affected by Syk inhibition is currently unknown. In TGF-β1 stimulated signaling pathways, TAK1 and MKK3/6 are the most upstream kinases of p38. Based on our results, expression of p-TAK1 and p-MKK3/6 was increased after TGF-β1 stimulation in NRK-49F cells, and Syk inhibition down-regulated the upstream kinases of p38. This is the first study to demonstrate the association between Syk and the TGFβ1-TAK1-MKK3/6-p38 signaling pathway.

5. Conclusions

In conclusion, we demonstrated that Syk inhibition reduces tubulointerstitial fibrosis in UUO mice in vivo and inhibits TGF-β1 induced kidney myofibroblast activation in vitro. Our results suggest that the effects of Syk inhibition on tubulointerstitial fibrosis may occur via two distinct mechanisms. The first is the down-regulation of innate immunity, contributing to decreased overall inflammation, and the second is the inhibition of TGF-β1-induced fibroblast-myofibroblast transformation mediated by decreased p38 phosphorylation. This study demonstrates the therapeutic potential of Syk inhibition for the treatment of renal tubulointerstitial fibrosis.

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Disclosure

No conflicts of interest, financial or otherwise, are declared by the authors.

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References


