SP600125, an inhibitor of c-Jun NH2-terminal kinase, blocks expression of angiotensin II-induced monocyte chemoattractant protein-1 in human mesangial cells

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Background: **We investigated the role of c-Jun NH2-terminal kinase (JNK), a member of the mitogen-activated protein kinase family, in the expression of angiotensin II (Ang II)-induced monocyte chemoattractant protein-1 (MCP-1) and transforming growth factor-1 (TGF-1), and in the production of fibronectin (FN), by human mesangial cells (HMCs).**

Methods: **JNK activation in cultured human mesangial cells was determined by Western blotting with an antibody against the phosphorylated Ser63 residue of c-Jun. Binding of the activator protein (AP-1) to the MCP-1 AP-1 motif was detected via the electrophoretic mobility shift assay (EMSA). The transient luciferase reporter was used to examine MCP-1 promoter activity; an RNase protection assay and ELISA were used respectively to detect the expression of MCP-1 mRNA and production of MCP-1, TGF-β and FN.**

Results: **Anthra (1,9-cd) pyrazol-6(2H)-one (SP600125), a pharmacological inhibitor of JNK, almost completely abolished Ang II-induced Ser63 phosphorylation of c-Jun at concentrations of 5-20 μmol/L: JNK activity was reduced by 75% with 10 μmol/L SP600125, and by 90% with 20 μmol/L. Ang II increased AP-1 binding to the MCP-1 AP-1 motif in a time-dependent manner, as detected by EMSA, while SP600125 effectively blocked this increased AP-1 binding in a concentration-dependent manner. Treatment with 100 nmol/L Ang II led to a steady increase in MCP-1 mRNA expression, and to an enhanced production of MCP-1, TGF-β and FN. These effects were blocked by SP60025 in a dose-dependent manner. SP600125 also reduced MCP-1 mRNA stability: the half-life of MCP-1 mRNA was approximately 5 hours in cells treated with Ang II only, but was reduced to 2 hours when treated with a combination of Ang II and SP600125.**

Conclusions: **These results show that the JNK/AP-1 pathway is involved in the expression of MCP-1 and TGF-β, and in extracellular matrix production. JNK is an important therapeutic target for glomerulonephritis and glomerulosclerosis.**

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Key words: **c-Jun NH2-terminal kinase; mesangial cells; monocyte chemoattractant protein-1; SP600125**

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Introduction

Mesangial cells (MCs) are prominent in the renal glomerulus, and participate actively in the renal inflammatory process. Upon stimulation with inflammatory factors or immune complexes,[1] MCs are activated and produce large amounts of proinflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), profibrogenic cytokines such as transforming growth factor-β (TGF-β), chemokines such as IL-8 and monocyte chemoattractant protein-1 (MCP-1), and extracellular matrix (ECM) proteins such as fibronectin.[1-3] Activation of MCs is almost invariably associated with various forms of glomerular renal diseases.

The octapeptide hormone angiotensin II (Ang II) is also involved in the inflammatory process following renal cell injury.[1,4] Ang II activates MCs, stimulating cell growth, hypertrophy and the synthesis of proinflammatory mediators and ECM components. Ang II acts through two specific receptors, angiotensin type 1 (AT1) and AT2. AT1 signaling regulates cell proliferation and the production of cytokines and ECM proteins, while signaling via AT2 regulates renal

natriuresis, inhibition of mesangial cell growth, and infiltration of renal inflammatory cells.[5] Ang II, via AT1, activates various nuclear transcription factors, including the activator protein-1 (AP-1), the STAT family of transcription factors, and the cAMP-response element-binding protein (CREB); Ang II also increases calcium release, and activates various protein kinases, including protein kinase C (PKC), protein tyrosine kinases (PTKs), and mitogen-activating protein kinases (MAPKs).[6,7] MAPKs are a family of serine/threonine protein kinases that play a key role in intracellular signaling pathways and link extracellular signals to intracellular regulatory proteins.[1,8]

MAPK family members, extracellular signal-regulated kinases 1/2 (ERK1/2) and p38 MAPK, participate in pathways that regulate the PDGF-induced MC proliferation and TGF-β1 and MCP-1 expression.[9] A third signal transduction pathway that involves the MAPK family is the c-Jun NH2-terminal kinase (JNK) pathway, which is also activated primarily by cellular stress and cytokines; its downstream targets include transcription factors that are important for cytokine expression.[1,10]

The role of the JNK pathway in MCP-1 and TGF-β1 expression in renal epithelial cells, endothelial cells, fibroblasts, and erythroid cells has been previously investigated using biochemical inhibitior of JNK.[11] In human MCs (HMCs), transfection with a dominant-negative JNK construct inhibits PDGF-induced proliferation, MCP-1 and TGF-β1 expression,[9] whereas in human vascular smooth muscle cells, transfection with dominant-negative JNK has no such effect.[12] SP600125 is a recently discovered pharmacological inhibitor of JNK that can be a useful tool in evaluating the role of JNK activity in physiological processes;[13,14] a recent report shows that SP600125 reduces Ang II-induced thrombospondin-1 expression in HMCs.[15] However, the specific effects of SP600125 on MCP-1 and TGF-β expression and on ECM production are unclear.

The aim of this study was to investigate the role of the JNK signaling in regulating the activated inflammatory response in glomerular mesangial cells. We used SP600125 as a pharmacological tool to inhibit the JNK pathway and studied the resulting effects on Ang II-induced MCP-1 and TGF-β expression, and on ECM production in HMCs. Our results show that JNK is an important factor in the regulation of MCP-1 and TGF-β expression in HMCs.

Methods

Plasmids and materials

The MCP-1-luciferase reporter was kindly provided by professor Atsuko Momoi at Kagawa Medical University.[16] SP600125 and N^1-methyl-substituted pyrazolanthrone (N^1-methyl-1,9-pyrazoloanthrone) were purchased from Calbiochem (San Diego, CA). Rabbit polyclonal anti-phospho-c-Jun (Ser63) antibody was purchased from Cell Signaling Technology, Inc. (Beverly, MA), and mouse monoclonal anti-cyclin D1, cyclin A, and β-actin antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). All other reagents were from Sigma Chemical Co. (St. Louis, MO).

Culture of human mesangial cells

Normal-appearing portions of human kidneys that were surgically removed from renal carcinoma were used to culture mesangial cells from collagenase-treated glomeruli. HMCs were established and characterized as previously reported.[1,10] Cells were grown to confluency in RPMI 1640 buffered with 10 mmol/L HEPES to pH 7.4 and supplemented with 20% fetal bovine serum, 5 μg/ml insulin and transferrin, 100 U/ml penicillin, and 100 mg/ml streptomycin. For cell passing, confluent cells were washed with phosphate buffered saline (PBS), removed with 0.025% trypsin/0.5 mmol/L ethylenediaminetetra-acetate (EDTA) in PBS, and plated in supplemented RPMI 1640 media. Experiments in this study were performed on cells between the 5th and 10th passages.

Western blotting analysis

At indicated time points, HMCs were rapidly washed with ice-cold PBS and were lysed for 10 minutes on ice in lysis buffer (50 mmol/L Tris, pH 7.5, 40 mmol/L NaCl, 1% Triton X-100, 2 mmol/L EDTA, 1 μg/ml leupeptin, 2 mmol/L DTT, and 1 mmol/L PMSF). Lysates were cleared by centrifugation at 14 000 × g (4°C) for 10 minutes. Total protein was quantified by the Bradford assay. Equal amounts of lysates were fractionated by 10% SDS-PAGE and electro-transferred onto Bio-Blot nitrocellulose membranes (Bio-Rad). Membranes were blocked for 1 hour at room temperature in TBS-T (20 mmol/L Tris-base pH 7.6, 150 mmol/L NaCl, 0.1% Tween-20) containing 5% bovine serum albumin, incubated with primary antibody in the blocking solution at 4°C overnight. Subsequently, the membranes were incubated with HRP-conjugated secondary Ab (diluted 1:1000) at room temperature for 1 hour. Antibody binding was visualized with an ECL kit (Amersham) and the chemiluminescent signal was quantitated using UVP software.

Transient luciferase reporter assay

HMCs were transfected by a conventional cationic liposome transfection method (Lipofectamine; Life Technologies, MD) with a luciferase reporter construct

fused to human MCP-1 promoter.[16] SP600125 and Ang II were added to the media 15 hours post-transfection; the cells were harvested and lysed 24 hours later, and luciferase activity was assayed using the Luciferase Reporter Assay System (Promega, Madison, WI) according to the manufacturer's protocol. For each transfection, 1 ng of Renilla luciferase reporter pRL-TK was included to normalize for differences in transfection efficiency.

Electrophoretic mobility shift assay (EMSA)

At indicated time points, HMCs were rapidly washed with ice-cold PBS, pelleted (500 × g, 10 minutes, 4°C), resuspended in hypotonic buffer (10 mmol/L HEPES, pH 7.9, 10 mmol/L KCl, 0.1 mmol/L EDTA, 0.1 mmol/L EGTA, 1 mmol/L DTT, 0.5 mmol/L PMSF, complete protease inhibitor cocktail tablets), and incubated for 10 minutes on ice. The cells were lysed by the addition of Nonidet P-40 (final concentration of 0.1%). The nuclear pellet was collected by centrifugation (14 000 × g, 10 minutes, 4°C), resuspended in extraction buffer (20 mmol/L HEPES, pH 7.9, 0.4 mmol/L KCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 1 mmol/L DTT, 1 mmol/L PMSF, complete protease inhibit cocktail tablets) and incubated for 10 minutes on ice. The tubes were centrifuged again (14 000 × g, 10 minutes, 4°C) and the supernatants were stored at -70°C until use. Protein content of the nuclear extracts was measured by the Bradford method (Bio-Rad, Hercules, CA).

For EMSA, double-stranded oligodeoxynucleotide probes for the consensus sequences of AP-1 binding sites of human MCP-1 gene promoter (-129 bp to -112 bp) (5'-TCCTGCTTGACTCCGCCC-3') were generated. The AP-1 probes were end-labeled with [γ^{32}P]ATP using T4-polynucleotide kinase. Nuclear extracts (20 μg) were incubated with 1.0 × 10^5 cpm of the labeled AP-1 probes for 30 minutes at room temperature in 20 μl binding buffer containing 10 mmol/L HEPES (pH=7.9), 50 mmol/L NaCl, 1 mmol/L DTT, 10% glycerol, 0.5 mmol/L EDTA, and 75 μg/ml of poly(dI-dC)/(dI-dC). All reaction mixtures were analyzed on a 6% polyacrylamide gel run in 0.25 × TBE (45 mmol/L Tris borate, 1 mmol/L EDTA); the gel was dried and visualized by autoradiography.

RNase protection assay

At indicated time points, HMCs were rapidly washed with ice-cold PBS. Total RNA was prepared with a TRIzol kit (Invitrogen, Carlsbad, CA, USA) as specified by the manufacturer, and was quantified spectrophotometrically. Human MCP-1 mRNAs were detected with an RNase protection assay (RPA) system from Pharmingen (San Diego, CA). Nuclease-protected RNA fragments were resolved on a 5% polyacrylamide sequencing gel. Bands were observed after autoradiography and band intensities were analyzed by the scanning of the images and the use of UVA software. The densitometric intensity was normalized with respect to the intensities of the band for the housekeeping gene for glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

MCP-1, TGF-β, and FN ELISA

At indicated time points, the medium was collected to quantify the levels of MCP-1, TGF-β, and FN protein excretion. MCP-1, TGF-β, and FN were measured using a commercial solid phase quantitative sandwich ELISA kit (Biosource International, Inc, Camarillo, CA) according to the manufacturer's instructions. According to the Biosource International, the ELISA kits are specific for human MCP-1, TGF-β1, and FN, and are sensitive to 8 pg/ml.

Statistical analyses

Results were presented as the mean ± SEM from three to four separate experiments. Statistical comparisons between multiple groups were performed by ANOVA using SPSS10.0. A minimum value of $P<0.05$ was considered statistically significant.

Results

SP600125 blocks Ang II-induced JNK activation

Our previous study showed that Ang II stimulated JNK activation in MCs.[1] In the present study we first tested the ability of SP600125 to inhibit JNK activity as measured by the phosphorylation of c-Jun residue Ser63. SP600125 inhibited Ang II-induced Ser63 phosphorylation of c-Jun in a concentration-dependent manner (Fig. 1A): JNK activity was reduced by 75% at 10 μmol/L and by 90% at 20 μmol/L. To clarify the specific blocking of the AT1 receptor-JNK pathway by SP600125, HMCs were treated with the AT1 receptor antagonist losartan (LOS, 10 μmol/L) or the AT2 receptor antagonist PD123319 (PD, 10 μM) for 30 minutes and then with 100 nmol/L Ang II for further 30 minutes. As shown in Fig. 1B, Ang II induced JNK activation was completely blocked by LOS, but not PD123319, indicating involvement of AT1, but not AT2 receptors.

SP600125 decreases transcription factor binding to the MCP-1 AP-1 motif

It has been previously reported that JNK regulates MCP-1 expression, in part, through the binding of transcription factor AP-1 to the AP-1 motif at -129 in the promoter region of the MCP-1 gene.[17] Since SP600125 inhibited JNK-dependent phosphorylation

and transcriptional activity of c-Jun, we hypothesized that SP600125 decreases AP-1 binding to the MCP-1 AP-1 motif. To test this hypothesis we used EMSA to detect the temporal response of AP-1 binding to the MCP-1 AP-1 motif after addition of Ang II. Nuclear extracts from HMCs treated with Ang II showed increased binding to the AP-1 motif in a time-dependent manner (Fig. 2A) while SP600125 effectively eliminated this effect in a dose-dependent manner (Fig. 2B).

SP600125 inhibits activation of the MCP-1 promoter by Ang II

The 5'-flanking region of the MCP-1 gene contains multiple AP-1 sites,[17] suggesting potential roles for AP-1 in the regulation of MCP-1 expression. Because SP600125 blocks AP-1 binding to the MCP-1 AP-1 motif, we speculated that SP600125 may inhibit the activation of the MCP-1 promoter. To examine this possibility, HMCs were transiently transfected with a MCP-1 promoter-driven luciferase reporter, and treated with Ang II in the absence, or presence, of SP600125. As shown in Fig. 3A, Ang II strongly increased the reporter activity, and treatment of the HMCs with SP600125 reduced the activity stimulated by Ang II in a dose-dependant manner. Ang II-increased reporter activity was inhibited by losartan, but not PD123319 (Fig. 3B). Taken together, these data indicate the involvement of AT1 receptor in Ang II-

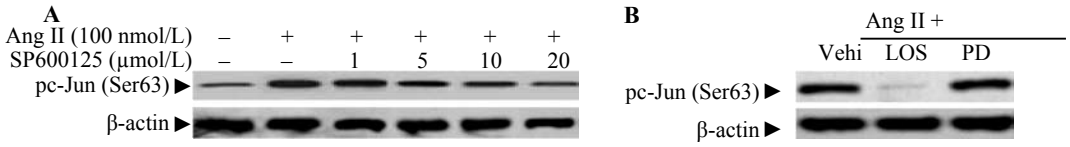

Fig. 1. Effect of SP600125 on JNK activity. **A:** HMCs were treated at various concentrations (1-20 μmol/L) of SP600125 for 30 minutes before stimulation with Ang II (100 nmol/L). Incubations were terminated after an additional 30 minutes; **B:** HMCs were treated with the AT1 receptor antagonist losartan (LOS, 10 μmol/L) or the AT2 receptor antagonist PD123319 (PD, 10 μmol/L) for 30 minutes and then with 100 nmol/L Ang II for further 30 minutes. JNK activity was detected by Western blotting, using antibodies against Ser63-phosphorylated c-Jun (pc-Jun).

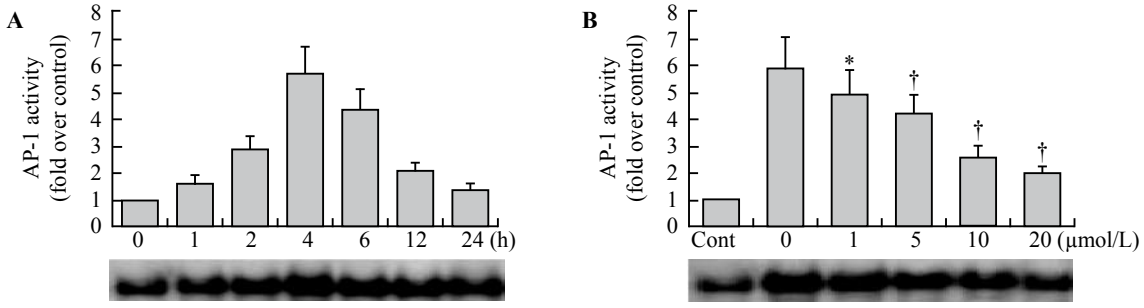

Fig. 2. Effect of SP600125 on the binding of transcription factor to the MCP-1 AP-1 motif. **A:** HMCs were treated with Ang II (100 nmol/L) for the indicated length of time; nuclear extract was prepared for EMSA with a nonradioactive oligonucleotide spanning the MCP-1 AP-1 motif. Results are expressed as fold increase over controls (represented as 1), and are shown as mean ± SEM from three independent experiments; **B:** HMCs were pretreated with various concentration of the SP600125 (1-20 μmol/L) for 30 minutes, then supplemented with Ang II (100 nmol/L) for 4 hours. Nuclear extracts were generated from the cells, and were analyzed by EMSA. Results are expressed as fold increase over controls (represented as 1), and are shown as mean ± SEM from three independent experiments. *: $P<0.05$; †: $P<0.01$, compared with cells treated with Ang II only.

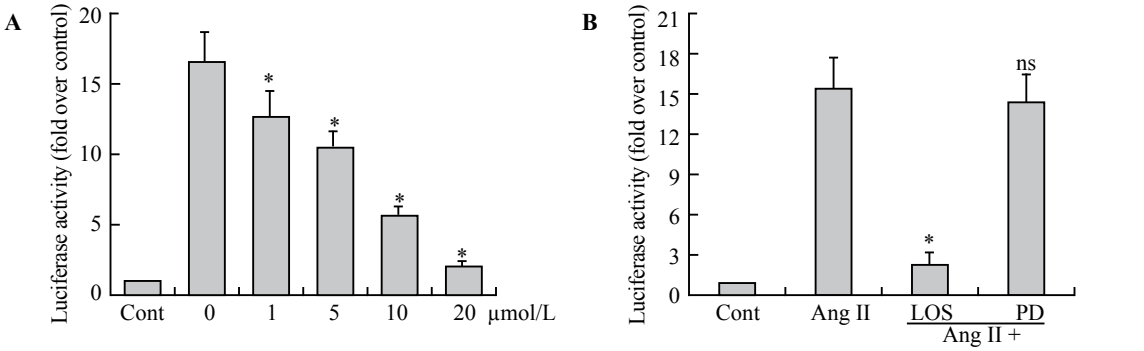

Fig. 3. Effect of SP600125 on the activation of the MCP-1 promoter. HMCs were transiently transfected with 10 μg of pMCP-Luc. After 15 hours, the cells were treated with SP600125 (1-20 μmol/L) for 30 minutes. Subsequently, all dishes were treated with 100 nmol/L Ang II with or without SP600125 (**A**) or AT1 receptor antagonist losartan (LOS, 10 μmol/L) or the AT2 receptor antagonist PD123319 (PD, 10 μmol/L) (**B**) for an additional 24 hours; the cells were harvested, and assayed for luciferase activity after normalizing for differences in transfection efficiency. The data are representative of triplicate experiments; *: $P<0.01$ compared with cells treated with Ang II only; ns: no significant with cells treated with Ang II only.

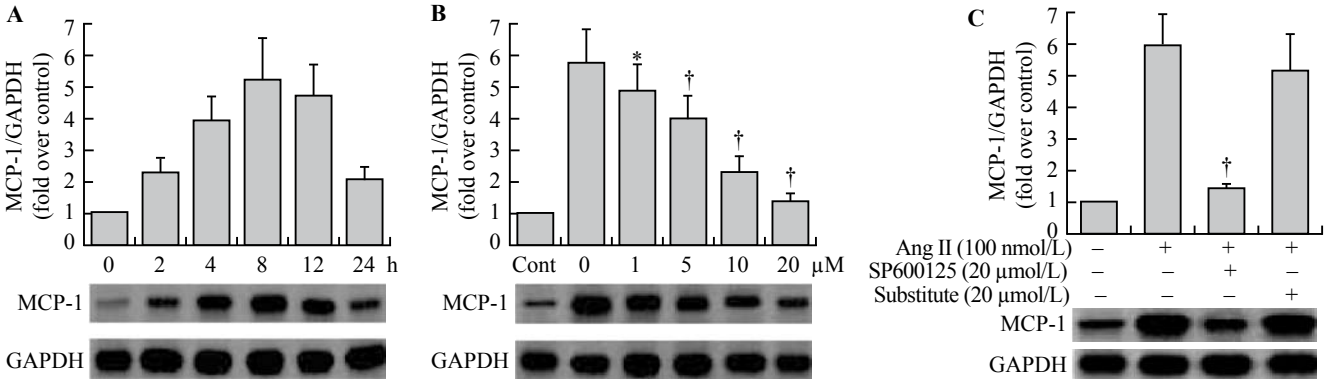

Fig. 4. Effects of SP600125 on Ang II-induced MCP-1 expression. **A:** MCs were incubated with Ang II (100 nmol/L) for different lengths of time as indicated, and MCP-1 mRNA expression was measured by RNA protection assay (RPA). **B:** MCs were incubated with various concentrations of SP600125 for 30 minutes prior to stimulation with Ang II (100 nmol/L). After 8 hours of incubation, MCP-1 mRNA expression was detected by RPA. **C:** MCs were pretreated with SP600125 (20 μmol/L) or a substitute (N^1-methyl-1,9-pyrazoloanthrone) for 30 minutes, then stimulated with Ang II (100 nmol/L). After incubating for 8 hours, expression of MCP-1 mRNA was measured by RPA; MCP-1 mRNA levels were normalized against GAPDH. Results are expressed as fold increase over controls, and are shown as mean ± SEM from three independent experiments. *: $P<0.05$; †:$P<0.01$ compared with cells treated with Ang II only.

induced JNK and MCP-1 promoter activation and the specific blocking of the AT1 receptor-JNK pathway by SP600125.

SP600125 decreases Ang II-induced MCP-1 mRNA expression

To investigate the role of JNK in Ang II-induced MCP-1 expression, HMCs were first stimulated with Ang II (100 nmol/L) for different periods of time to determine the time-response. A representative autoradiogram of a gel (Fig. 4A) showed that following Ang II treatment, MCP-1 expression changed in a time-dependent manner, first detectable at 2 hours, peaking at 8 hours and gradually declining thereafter. Examination of the effect of SP600125 on this Ang II-induced MCP-1 expression revealed that SP600125, when applied at 1 μmol/L to 20 μmol/L, had a concentration-dependent inhibitory effect on Ang II-induced MCP-1 mRNA expression (Fig. 4B). To test whether this inhibitory effect was related to JNK inhibition, we used N^1-methyl substituted pyrazolanthrone as a control compound, which is structurally related to SP600125 but is more than 100-fold less potent in inhibiting JNK. N^1-methyl-1,9-pyrazoloanthrone (20 μmol/L) inhibited MCP-1 mRNA expression slightly, but the effect was considerably less potent than the effect of SP600125 at the same concentration (Fig. 4C).

SP600125 decreases Ang II-induced MCP-1 mRNA stability

MCP-1 mRNA expression can be regulated at the transcriptional or post-transcriptional level. To study whether the inhibitory effect on MCP-1 gene expression by SP600125 is also caused by a decrease in mRNA

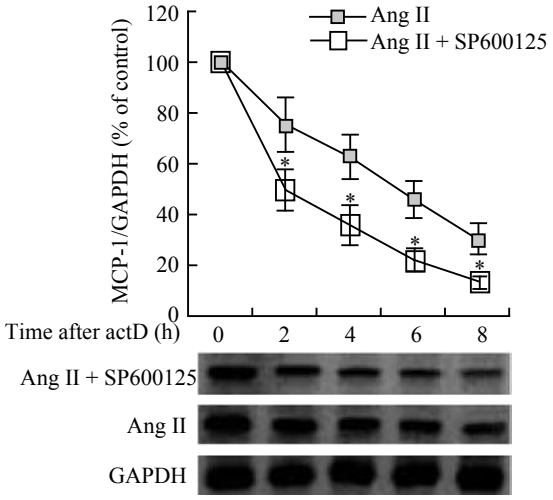

Fig. 5. Effect of SP600125 on the stability of MCP-1 mRNA. HMCs were incubated with or without SP600125 (20 mol/L) for 30 minutes before stimulation with Ang II (100 nmol/L). Actinomycin D (actD) (10 μg/ml) was added to the cells after an additional 6 hours of Ang II stimulation. Incubations were terminated, and total RNA isolated, at indicated time points following addition of actinomycin D. MCP-1 and GAPDH mRNA was measured by RPA. MCP-1 mRNA levels were normalized against GAPDH. Results are expressed as percentage of controls, and are shown as mean ± SEM from three independent experiments. *: $P<0.05$; †: $P<0.01$, compared with controls.

stability, HMCs were pretreated with Ang II for 6 hours, followed by incubation in actinomycin D (a transcription inhibitor), for a further 0, 2, 4, 6, or 8 hours. As indicated in Fig. 5, when transcription was blocked with actinomycin D, the relative levels of MCP-1 mRNA decreased more rapidly in SP600125-treated cells compared with untreated cells, indicating that SP600125 reduced mRNA stability. The half-life of MCP-1 mRNA was approximately 5 hours in cells treated with Ang II

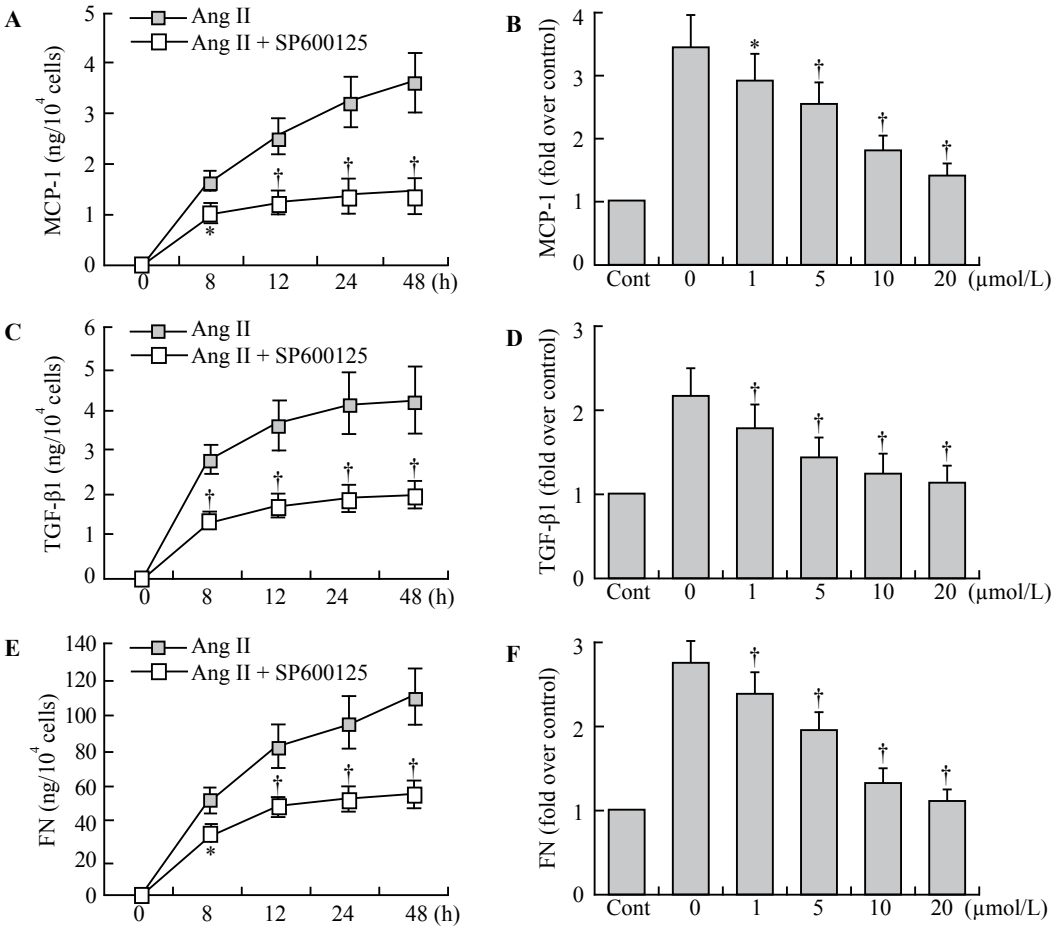

Fig. 6. Effect of SP600125 on Ang II-induced MCP-1, TGF-β1 and FN production. HMCs were preincubated with or without SP600125 (10 μmol/L) for 30 minutes, then incubated with Ang II (100 nmol/L) for the indicated time periods (**A**, **C**, **E**), or cells were pretreated with or without various concentrations of SP600125 (1-20 μmol/L) for 30 minutes, and then incubated with Ang II (100 nmol/L) for 24 hours (**B**, **D**, **F**); MCP-1, TGF-β1 and FN protein were measured by ELISA. Results from four independent experiments are shown as mean ± SEM. *: $P<0.05$; †: $P< 0.01$, compared with cells treated with Ang II only respectively.

only but was reduced 2 hours when the cells were treated with a combination of Ang II and SP600125.

SP600125 blocks Ang II-induced MCP-1, TGF-β and FN production

MCP-1, TGF-β and FN proteins were measured in the supernatants of cultured MC cells that were stimulated with Ang II (100 nmol/L) with or without SP600125. MCP-1, TGF-β and FN proteins were constitutively produced by MCs and the production was significantly stimulated for 8 to 48 hours after addition of Ang II (Fig. 6). Preincubation of cells with SP600125 (20 μmol/L) significantly inhibited Ang II-induced MCP-1, TGF-β and FN production during this time period. We also measured MCP-1, TGF-β and FN proteins in supernatants from cells cultured for 24 hours in the presence of Ang II with or without various concentrations of SP600125 (1-20 μmol/L). SP600125 inhibited Ang II-induced production of MCP-1, TGF-β and FN in a concentration-dependent manner.

Discussion

We demonstrated that the JNK-specific inhibitor SP600125 inhibits Ang II-induced MCP-1 and TGF-β expression and FN production in HMCs by suppressing JNK-dependent phosphorylation of c-Jun as well as by blocking the binding of AP-1 to the MCP-1 promoter. Because of MC proliferation, and production of chemokines, profibrogenic cytokines, ECM components play a direct role in the progression to glomerular inflammation and glomerulosclerosis,[1-3] while SP600125 may have therapeutic potential in the treatment of inflammatory glomerular diseases and glomerulosclerosis.

We have shown that SP600125 inhibits Ang II-induced c-Jun phosphorylation in HMCs with an IC_{50} of 5 to 10 μmol/L, which is similar to the IC_{50} value

detected in Jurkat T cells.[13] In that same report, partial inhibition of other MAPK pathways was observed only when SP600125 was used at concentrations greater than 25 μmol/L.[13] The effects of SP600125 on MCP-1 expression that were observed in the present study were evident at concentrations of 10 to 20 μmol/L; we did not observe an inhibitory effect on other MAPKs at these concentrations.[1] Furthermore, N^1-methyl-1,9-pyrazoloanthrone, which is chemically related to SP600125 but is more than 100-fold less potent in inhibiting JNK, had only a minimal inhibitory effect on Ang II-induced MCP-1 expression when used at a 20 μmol/L concentration. These results suggest that the effect of SP600125 on Ang II-induced MCP-1 expression was specific to the inhibition of JNK.

MCP-1 is a member of the chemokine family and specifically attracts monocytes. In response to inflammatory stimuli, MCP-1 is ubiquitously produced by various cell types including resident glomerular cells.[18] In glomerulonephritis, infiltration of monocytes/macrophages is a common pathologic feature. Expression of MCP-1 is observed in the mesangium of inflamed glomeruli while MCP-1 produced by MCs plays a crucial role in macrophage attraction during glomerular inflammation.[18,19] Previous reports have shown that the 5'-flanking region of the MCP-1 gene contains NF-κB sites and AP-1 sites.[20] The roles of NF-κB and AP-1 in the regulation of MCP-1 expression are both stimulus-specific and tissue-specific. In MCs, IL-1β-induced MCP-1 expression is mediated by NF-κB and AP-1,[20] while proteasome inhibitor-induced MCP-1 expression is mediated by the JNK/AP-1 pathway.[17] However, whether Ang II-induced MCP-1 expression is mediated by AP-1 in MCs has not been clearly determined. In our study, Ang II stimulated JNK and AP-1 activity in a time-dependent manner; by using the JNK-specific inhibitor SP600125, we explored the role of JNK/AP-1 in Ang II-induced MCP-1 production. When MCs were preincubated with SP600125, Ang II-induced MCP-1 production was inhibited in a concentration-dependent manner. It has been reported that SP600125 induced inducible nitric-oxide synthase (iNOS)[21] and cyclooxygenase-2 (COX-2)[22] mRNA degradation in macrophages. Consistent with these studies, our data showed that SP600125 treatment facilitated MCP-1 mRNA degradation in HMCs, reducing its half life from 5 to 2 hours. These results indicate that JNK/AP-1 activation is involved in Ang II-induced MCP-1 expression.

TGF-β is a well-known multifunctional cytokine produced by a variety of cell types, including monocytes, macrophages, lymphocytes, fibroblasts, endothelial cells, and MCs.[23-25] Previous studies have demonstrated an important role for TGF-β in renal disease: it appears to be the major cytokine in regulating renal residential cell proliferation, differentiation, and angiogenesis, in increasing the synthesis of matrix proteins such as fibronectin and collagen types I and III, and in decreasing synthesis of proteases which could digest ECM molecules in various models.[23-25] TGF-β is also strongly implicated in the inflammatory process: it is released at the site of inflammation and promotes recruitment of leukocytes, including monocytes, and neutrophils, as well as their activation and cytokine synthesis.[23-25] Our results show that SP600125 strongly inhibits TGF-β expression and FN production in a dose-dependent manner. TGF-β reportedly induces FN production in MCs.[26] We investigated whether SP600125 inhibited FN production through reducing TGF-β expression. By using an anti-TGF-β antibody, we demonstrated that SP600125 has a direct inhibitory effect on FN production (data not shown). Our work suggests that SP600125 might have antifibrogenic properties due to its ability to downregulate the synthesis of profibrogenic cytokines such as TGF-β and FN.

In summary, this is the first study to show that SP600125, an inhibitor of JNK, reduces Ang II-induced MCP-1 and TGF-β expression and FN production. Recently, a therapeutic effect of SP600125 in inflammatory arthritis has been documented in a rat adjuvant arthritis model. This anti-inflammatory effect, combined with the present findings, indicates that JNK is an important therapeutic target for glomerulonephritis and glomerulosclerosis.

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Competing interest: None.
Contributors: Zhang AH, Huang SM, and Chen RH designed the research. Zhang AH, Ding GX, and Pan XQ performed the experiments. Zhang AH and Huang SM wrote the manuscript.

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