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Tranilast inhibits interleukin-1β-induced monocyte chemoattractant protein-1 expression in rat mesangial cells

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Abstract

Monocyte chemoattractant protein-1 (MCP-1), a member of the CC subfamily of chemokines, plays a crucial role in the progression of glomerulonephritis by recruitment of monocytes. Tranilast, a clinically used anti-allergic drug, has been demonstrated to have various anti-inflammatory and anti-proliferative effects, and recently has been reported to prevent restenosis after percutaneous transluminal coronary angioplasty. In this study, we investigated whether tranilast inhibits MCP-1 secretion in mesangial cells. Tranilast inhibited interleukin-1β-induced MCP-1 secretion and mRNA expression in a concentration-dependent manner. Luciferase assay showed that tranilast suppressed interleukin-1β-induced nuclear factor-κB (NF-κB)-dependent transcription. Interleukin-1β-induced Jun N-terminal kinase (JNK) activation was also suppressed selectively by tranilast. These results indicate that tranilast inhibits interleukin-1β-induced MCP-1 production, at least in part, by inhibiting NF-κB activity and that suppression of JNK activation might be involved in the inhibition of MCP-1 production. Tranilast may serve as a new therapeutic agent for glomerulonephritis through anti-chemokine property.

Keywords: MCP-1 (monocyte chemoattractant protein-1); Tranilast; Mesangial cell; NF-κB (nuclear factor-κB)

1. Introduction

Monocyte infiltration of glomeruli is a common pathologic feature of glomerulonephritis (Atkins, 1998). Monocyte chemoattractant protein-1 (MCP-1), a member of the CC-chemokine subfamily, recruits and activates mainly monocyte/macrophages in inflammatory sites (Luster, 1998). In human renal disorders, MCP-1 expression was detected in inflamed glomeruli and correlated with the degree of glomerular macrophage accumulation (Rovin et al., 1994; Segerer et al., 2000). Urinary MCP-1 secretion in glomerulonephritis patients correlates well with disease activity and has the potential to be utilized as an indicator of therapeutic efficacy (Noris et al., 1995; Rovin et al., 1996). In experimental models of renal disease, administration of an antibody-neutralizing MCP-1 attenuated macrophage infiltration and glomerulosclerosis. (Tang et al., 1996; Wenzel et al., 1997). Together with the findings that resident glomerular cells, including mesangial cells, can produce MCP-1, these reports support the concept that locally produced MCP-1 plays a central role in the progression of glomerulonephritis. Although intervention targeting MCP-1 has emerged as a new therapeutic approach, practical pharmacologic agents, which inhibit MCP-1 expression, are as yet limited.

Tranilast, *N*-(3,4-demethoxycinnamoyl)-anthranilic acid, has been used clinically to treat allergic diseases, such as atopic dermatitis, bronchial asthma and allergic rhinitis. It has also been shown that tranilast suppresses hypertrophic scarring and keloid formation by inhibiting fibroblast proliferation and collagen synthesis (Isaji et al., 1987). Recently, it was reported that tranilast dramatically reduced the restenosis rate of percutaneous transluminal coronary angioplasty in Japanese patients (TREAT study, Tamai et al., 1999). A multicenter study of 11,500 patients is also being conducted in a Western population to confirm the effect of tranilast in reducing restenosis and cardiovascular events after coronary intervention (PRESTO study, Holmes et al., 2000). We previously reported that tranilast suppresses the effects of platelet-derived growth factor on

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the proliferation of and inhibition of nitric oxide synthase induction in vascular smooth muscle cells (Hishikawa et al., 1996). Moreover, tranilast suppresses the release of cytokines from inflammatory cells and inhibits collagen synthesis in vascular smooth muscle cells (Suzawa et al., 1992; Fukuyama et al., 1996). As for the effects on chemokine expression, tranilast has been recently shown to inhibit interleukin-8 release from monocytes (Capper et al., 2000). It is conceivable that these various effects of tranilast contribute to the prevention of restenosis after percutaneous transluminal coronary angioplasty. In the field of renal parenchymal cells, however, tranilast's actions had not been investigated until we demonstrated that tranilast inhibits proliferation of glomerular mesangial cells (Hishikawa et al., 1995). Based on the various aforementioned actions and clinical usefulness of tranilast, we hypothesized that tranilast can be utilized as a therapeutic agent for glomerulonephritis and focused on its action on MCP-1 expression in mesangial cells.

Cytokines, such as interleukin-1β, have been suggested to play crucial roles in the induction of MCP-1 in the progression of renal injury (Matsumoto, 1990; Yoshioka et al., 1993; Jenkins et al., 1994). In mesangial cells, interleukin-1β activates mitogen-activated protein (MAP) kinases, which are divided into three distinct subgroups: extracellular signal-regulated kinase (ERK) (Wilmer et al., 1997), p38 kinase, and Jun N-terminal kinase (JNK) (Guan et al., 1996). In mesangial cells, p38 kinase is reportedly necessary for the induction of MCP-1 expression by interleukin-1β (Rovin et al., 1999). In other cell types, all of the MAP kinases are suggested to contribute to the activation of nuclear factor-κB (NF-κB) (McGilvray et al., 1997; Wesselborg et al., 1997; Tuyt et al., 1999), which is a key transcriptional regulator of MCP-1 (Rovin et al., 1995; Stylianou et al., 1999).

In this study, we examined the effect of tranilast on MCP-1 expression in mesangial cells and also investigated how tranilast modulates the signaling pathways implicated in the induction of MCP-1.

2. Materials and methods

2.1. Materials

Recombinant human interleukin-1β was purchased from Genzyme/Techne (MNPS, MN). Tranilast was generously porvided by Kissei Pharmaceutical (Nagano, Japan). RPMI 1640 medium, Dulbecco's modified essential medium (DMEM) and fetal bovine serum were from Gibco (Grand Island, NY). Insulin–transferrin–selenite supplement was from Sigma (St. Louis, MO). Enzyme-linked immunosorbant assay (ELISA) kit for rat MCP-1 was form Biosource International (Camarillo, CA). A 578 bp fragment from the clone pcJE-1 containing the coding region for mouse MCP-1/JE (Rollins et al., 1988) was kindly provided by Dr. B.J. Rollins (Boston, MA) and the cDNA probe specific for the human GAPDH was from Clontech (Palo Alto, CA). Reporter plasmid pNF-κB-Luc (Wahl et al., 1998) was kindly provided by Dr. R.M. Schmid (Ulm, Germany) and pRL-TK vector was from Promega (Madison, WI). All other chemicals and reagents were obtained from commercial sources and were of reagent or molecular biology grade.

2.2. Culture of mesangial cells

Rat glomerular mesangial cells were cultured and characterized as previously described (Hirahashi et al., 2000). For experiments, mesangial cells from passages 5–12 were used. Cells were cultured at 37 °C in a humidified air atmosphere with 5% CO_2. RPMI 1640 medium, supplemented with 10% fetal bovine serum and an insulin–transferrin–selenite supplement, was used as the growth medium. After reaching confluence, cells were made quiescent by 48-h incubation with DMEM supplemented with 0.5% fetal bovine serum before all experiments.

2.3. Measurement of MCP-1 protein concentration

Mesangial cells cultured in 48-well plates pretreated with or without tranilast for 1 h were stimulated with interleukin-1β (0.2 nM) for 12 h. The supernatants were collected and used for quantification of MCP-1 with an ELISA kit according to the manufacturer's instructions.

2.4. Northern blot analysis

Mesangial cells cultured in 10 cm dishes pretreated with or without tranilast for 1 h were stimulated with interleukin-1β for 6 h. Total RNA was extracted, size-fractioned, and transferred to nylon membranes (Hybond: Amersham, Little Chalfont, UK) as described previously (Marumo et al., 1997). cDNA probes specific for mouse MCP and human GAPDH were labeled by the random priming method with [α-^{32}P] dCTP as previously described (Marumo et al., 1997). After hybridization with the probes and washing, the radioactivities of the blots were measured using an imaging analyzer (BAS2000, Fuji Photo Film, Tokyo, Japan).

2.5. MCP-1 mRNA stability

After cells had been stimulated with interleukin-1β for 5 h, tranilast was added to the culture medium and coincubated with interleukin-1β for 1 h. After the 1-h coincubation period, the transcription inhibitor actinomycin D (5 μg/ml) was added to the culture medium. Total RNA was extracted at the indicated time points after addition of actinomycin D. Northern blot analyses for MCP-1 mRNA and GAPDH mRNA were then performed as described above.

2.6. Transfection and luciferase assay

Transfections were performed with a Superfect kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Mesangial cells cultured in 24-well plates were transfected with 0.5 μg of pNF-κB-Luc (Wahl et al., 1998). Cotransfection with 0.16 μg of pRL-TK vector was used in all experiments to normalize transfection efficiency. After transfection and luciferase protein expression for 24 h, mesangial cells pretreated with or without tranilast were exposed to interleukin-1β for 6 h. Cells were then lysed, and luciferase activities were measured using the dual luciferase reporter assay system (Promega). All values were corrected for transfection efficiency by calculating the ratio of κB luciferase to pRL-TK luciferase activity.

2.7. Electromobility shift assay

Mesangial cells cultured in 6-cm dishes pretreated with or without tranilast for 1 h were stimulated with interleukin-1β for 1, 3 or 6 h. After treatment, cells were washed twice with ice-cold PBS and nuclear proteins were extracted as described previously (Hirahashi et al., 2000). As probes, double-stranded oligonucleotides containing the sequence of the binding site for NF-κB, (5′-AGT TGA GGG GAC TTT CCC AGG C-3′, Promega) were radiolabeled with 30 μCi[γ-^{32}P]ATP using a 5′ labeling kit (Promega). Nuclear proteins (6 μg) were incubated with 3000 counts of labeled oligonucleotide in a binding buffer for 20 min at room temperature. The reaction mixture was loaded on a native 6% polyacrylamide gel buffered with 89 mM Tris, 89 mM boric acid, and 2 mM EDTA and electrophoresed. After vacuum drying, the gels were exposed to X-ray film. When competition assay was done, nuclear extracts were preincubated for 10 min with 100 times amounts of unlabeled oligonucleotides. For supershift analysis, 1 μg of specific polyclonal anti-p65 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) or anti-p50 (Santa Cruz) antibody was added and incubated at 4 °C for 12 h before the addition of the labeled probe.

2.8. Western blot analysis for detection of phosphorylated MAP kinases

Activations of the ERK, p38 and JNK pathways were analyzed by Western blot with anti-phosphorylated ERK, p38 and JNK antibodies, respectively. After treatment, the confluent cells in 6 wells (5×10^5 cells/dish) were lysed in 100 μl of solution containing 10% glycerol, 2.3% sodium dodecyl sulfate, Tris–HCl (pH 6.8) 62.5 mM, 0.01% bromophenol blue and 5% mercaptoethanol. The lysates were then heated at 100 °C for 5 min. Whole cell lysates containing 20 μg of protein were subjected to 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis. The separated proteins were electrophoretically transferred to nitrocellulose membranes (Hybond, Amersham). The blots were blocked in blocking buffer (10 mM Tris–HCl (pH 7.5), 100 mM NaCl, 0.5% Tween-20, 5% nonfat dry milk) for 1.5 h and incubated with primary antibodies against anti-phosphorylated MAP kinase (dilution 1:1000; anti-phosphorylated ERK, anti-phosphorylated p38, anti-phosphorylated JNK) overnight at room temperature. They were then incubated with secondary antibody conjugated to horseradish peroxidase (dilution 1:1000; Amersham). Immunoreactive bands were visualized by chemiluminescence (ECL, Amersham). After detection of

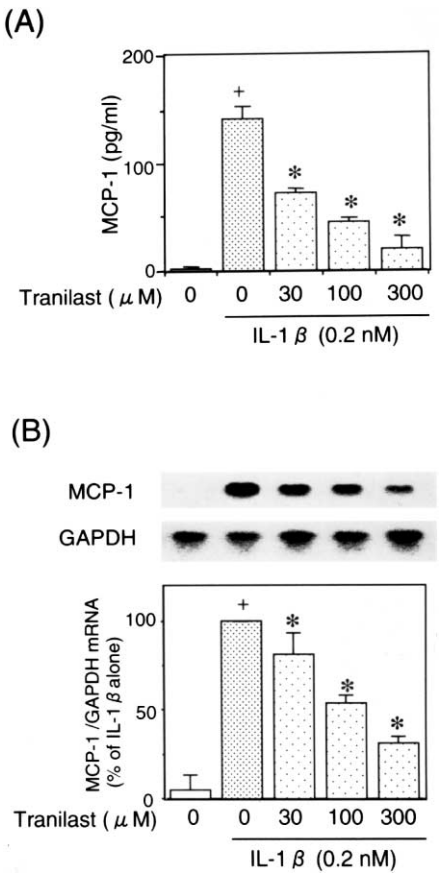

Fig. 1. (A) Inhibition of interleukin-1β (interleukin-1β)-induced monocyte chemoattractant protein-1 (MCP-1) secretion by tranilast. Mesangial cells were incubated with interleukin-1β (0.2 nM) alone or with serial concentrations of tranilast for 12 h and MCP-1 secretion was measured by ELISA. Mesangial cells were pretreated with tranilast for 1 h prior to interleukin-1β stimulation. Values are means ± S.E.M. ($n = 4$). + $P < 0.01$, vs. control. * $P < 0.05$, vs. interleukin-1β alone. (B) Suppression of interleukin-1β-induced MCP-1 mRNA by tranilast. Mesangial cells were incubated with interleukin-1β (0.2 nM) alone or with various concentrations of tranilast for 6 h and subjected to Northern blot analysis to detect MCP-1 mRNA expression. The radioactivity of each RNA sample hybridized to the MCP-1 probe was divided by that hybridized to the GAPDH probe. Mesangial cells were preincubated with tranilast for 1 h prior to interleukin-1β stimulation. A representative autoradiogram from four different experiments with identical results is shown. The corrected value for each lane was then divided by that of interleukin-1β alone and expressed as percentage of interleukin-1β alone. Values are means ± S.E.M. ($n = 4$). + $P < 0.01$, vs. control. * $P < 0.05$, vs. interleukin-1β alone.

a phosphorylated MAP kinase band, the membrane was stripped (strip buffer containing 100 mM β-mercaptoethanol, 2% SDS, 62.5 mM Tris–HCl pH6.7) and rehybridized with anti-MAP kinase antibody (dilution 1:1000; anti-ERK1, anti-p38 and anti-JNK1).

2.9. Statistics

Multiple comparisons were evaluated with analysis of variance, followed by Fisher's protected least significant difference method. Data are presented as means ± S.E., and P values < 0.05 were considered to be statistically significant.

3. Results

3.1. Inhibition of interleukin-1β-induced MCP-1 expression by tranilast

Under basal culture conditions (0.5% fetal bovine serum), mesangial cells secreted only small amounts of MCP-1 protein. Interleukin-1β induced marked MCP-1 protein production in mesangial cells. Since MCP-1 secretion peaked at the 0.2 nM interleukin-1β concentration, this concentration of interleukin-1β was employed for all experiments. After preincubation with tranilast for 1 h, mesangial cells were incubated with interleukin-1β in the presence of serial concentrations (30 to 300 μM) of tranilast for 12 h. Interleukin-1β-induced MCP-1 secretion was concentration dependently inhibited by tranilast (Fig. 1A). More than 95% of cells treated with 300 μM tranilast for 12 h were negative with trypan blue assay.

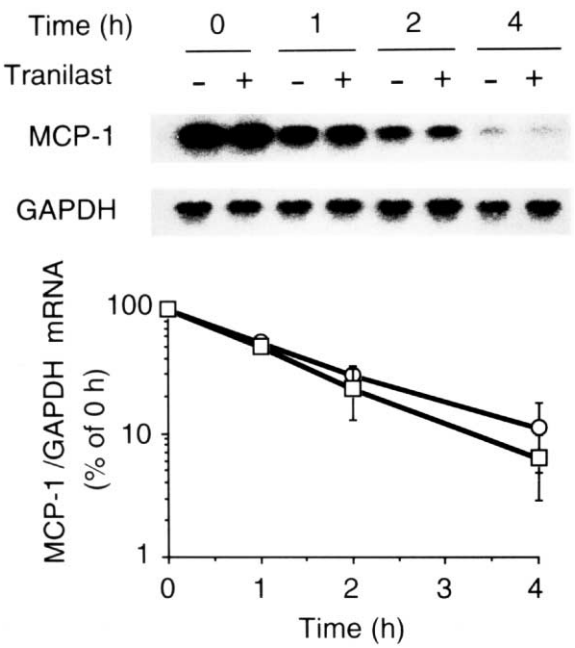

Fig. 2. Effect of tranilast on MCP-1 mRNA stability. Mesangial cells were stimulated with interleukin-1β (0.2 nM) for 5 h and then coincubated with vehicle or tranilast (100 μM) for 1 h. After the coincubation period, actinomycin D (5 μg/ml) was added to the culture medium and MCP-1 expression was examined by Northern blot analysis at 0, 1, 2 or 4 h after the addition of actinomycin D. A representative autoradiogram from four different experiments with identical results is shown. The radioactivity of each RNA sample hybridized to the MCP-1 probe was divided by that hybridized to the GAPDH probe. The corrected value (means ± S.E.M.) for each time point was then divided by the 0 h value and was plotted as a percentage of the 0 h value against time. Open circle and open square indicate interleukin-1β alone and tranilast, respectively.

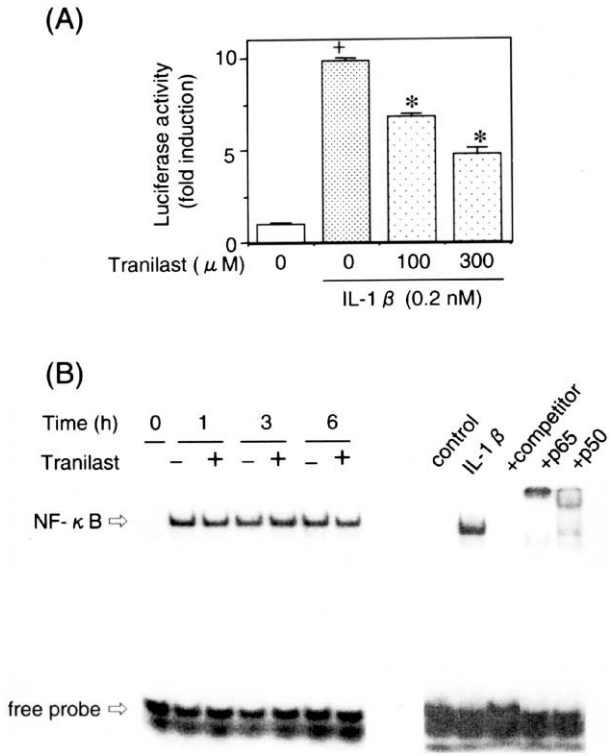

Fig. 3. (A) Effect of tranilast on interleukin-1β-induced nuclear factor-κB (NF-κB)-dependent transcription. Mesangial cells were transiently transfected with pNF-κB-Luc as described in Materials and methods. After the transfection, cells were stimulated with interleukin-1β (0.2 nM) alone or with tranilast (100 μM, 300 μM) for 6 h. Mesangial cells were preincubated with tranilast for 1 h prior to interleukin-1β stimulation. Cotransfection with pRL-TK vector was used in all experiments to normalize transfection efficiency. Luciferase activity was measured with the dual-luciferase reporter assay system and all values were corrected by calculating the ratio of κB luciferase to TK luciferase activity. Values are means ± S.E.M. ($n = 5$). + $P < 0.01$, vs. control. * $P < 0.05$, vs. interleukin-1β alone. (B) Effect of tranilast on interleukin-1β-induced DNA binding of NF-κB. Left panel shows time course of the interleukin-1β-induced DNA binding of NF-κB. Mesangial cells were stimulated with interleukin-1β (0.2 nM) alone or with tranilast (300 μM) for 1 h, 3 h or 6 h. Mesangial cells were preincubated for 1 h with tranilast prior to interleukin-1β stimulation. After incubation, nuclear extracts were obtained and analyzed by EMSA. A representative autoradiogram from four different experiments with identical results is shown. Right panel shows competition assay and supershift assays. For competition assay, nuclear extracts obtained from mesangial cells stimulated with interleukin-1β were preincubated with excess amount of unlabeled NF-κB consensus oligonucleotide (competitor). The extracts were pretreated with antibody against NF-κB subunit p50 or p60 for supershift assays.

Northern blot analysis revealed expression of MCP-1 mRNA in mesangial cells to be maximal after 6-h stimulation with interleukin-1β (data not shown). MCP-1 mRNA expression stimulated by interleukin-1β for 6 h was inhibited by coincubation with tranilast. Inhibition of MCP-1 mRNA expression by tranilast was also concentration dependent (Fig. 1B). Since tranilast did not decrease MCP-1 mRNA stability in the presence of actinomycin D, the inhibitory effect of tranilast on MCP-1 induction appears to be exerted at the transcriptional level (Fig. 2).

3.2. Effect of tranilast on NF-κB activity

Since NF-κB is reportedly one of the key transcriptional regulators of MCP-1 expression in mesangial cells stimulated by interleukin-1β (Rovin et al., 1995; Stylianou et al., 1999), we performed an NF-κB-induced luciferase activity assay to investigate the possibility that tranilast inhibits MCP-1 expression via suppression of NF-κB. As shown in Fig. 3A, tranilast suppressed NF-κB-driven luciferase activity induced by interleukin-1β. To examine the effect on DNA-binding activity of NF-κB, an electrophoretic mobility shift assay (EMSA) was performed. As shown in Fig. 3B, interleukin-1β induced DNA-binding activity of NF-κB with continuing activation to 6 h. Tranilast did not inhibit DNA-binding activity of NF-κB. The specificity of the NF-κB binding activity was demonstrated by the finding that an excess of unlabeled NF-κB consensus oligonucleotide prevented the formation of complexes which contain the labeled probe. Treatment of nuclear extracts with specific anti-p50 or anti-p60 antibody resulted in a supershift.

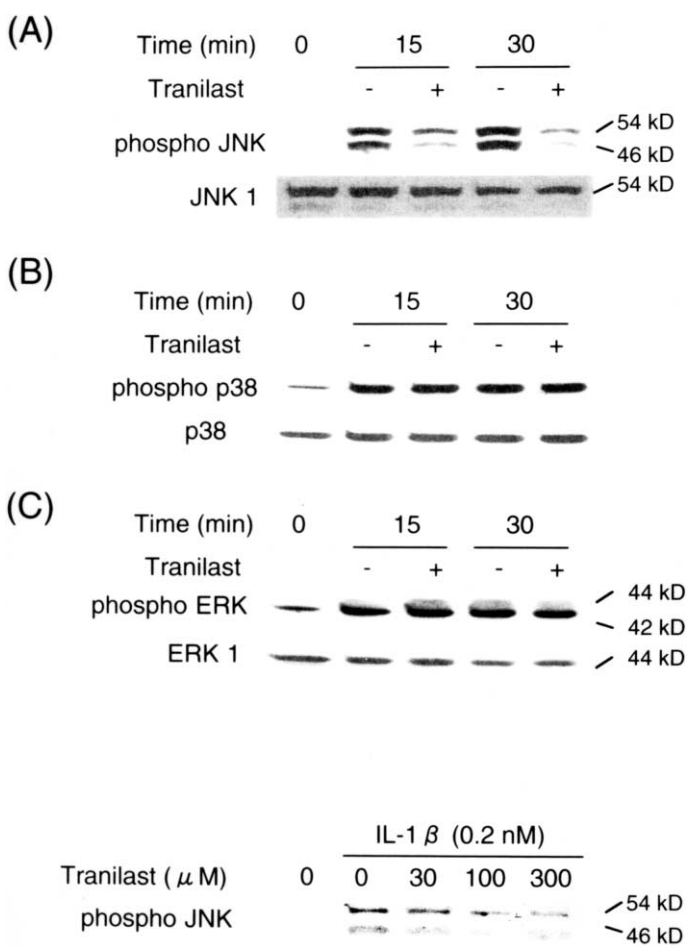

Fig. 4. (A) Effect of tranilast on interleukin-1β-induced phosphorylation of mitogen-activated protein (MAP) kinases. Mesangial cells were stimulated with interleukin-1β (0.2 nM) alone (−) or with tranilast (300 μM, +) for 15 and 30 min. Mesangial cells were preincubated with tranilast for 1 h prior to interleukin-1β stimulation. Whole cell lysates were obtained and subjected to Western blot analysis using anti-phosphorylated Jun N-terminal kinase (JNK) antibody (upper panel), anti-phosphorylated extracellular signal-regulated kinase (ERK) antibody (middle panel) and anti-phosphorylated p38 kinase antibody (lower panel). These membranes were reprobed with anti-JNK1 antibody, anti-ERK1 antibody and anti-p38 antibody, respectively, to assess protein expression. A representative autoradiogram from three different experiments with identical results is shown. (B) Concentration-dependent inhibition of tranilast on interleukin-1β-induced phosphorylation of JNK. Mesangial cells were stimulated with interleukin-1β (0.2 nM) alone or with serial concentrations of tranilast for 30 min and phosphorylated JNK in whole cell lysates was detected by Western blot analysis as described above. A representative autoradiogram from three different experiments with identical results is shown.

3.3. Effects of tranilast on the activities of MAP kinases

We next evaluated the effects of tranilast on interleukin-1β-induced activation of MAP kinases, which are suggested to contribute to NF-κB-dependent transcription. As shown in Fig. 4A, tranilast inhibited the phosphorylation of JNK activated by interleukin-1β. Although ERK and p38 were also activated by interleukin-1β, neither kinase was affected by tranilast treatment. To evaluate MAP kinase protein levels, stripped membranes were reprobed with antibodies against MAP kinases. Tranilast did not affect MAP kinase protein levels. The inhibitory effect of tranilast on JNK activation was concentration dependent (Fig. 4B).

4. Discussion

In this study, we demonstrated interleukin-1β-triggered induction of MCP-1 to be suppressed by tranilast in mesangial cells. Tranilast has been clinically used for treatment of allergic diseases and has been recently highlighted for its inhibitory effects on release or production of various chemical mediators and cytokines from inflammatory cells. To our knowledge, this is the first report showing the inhibitory effects of tranilast on MCP-1 production. Northern blot analysis showed that tranilast inhibited the induction of MCP-1 by interleukin-1β at the mRNA level and luciferase assay clearly demonstrated that tranilast inhibited interleukin-1β-triggered NF-κB activation in mesangial cells. When a standard clinical dose of tranilast (300 mg/day) is administered to humans, the plasma concentration of tranilast exceeds 100 μM (Tanaka et al., 1994), which was enough to suppress NF-κB activation and MCP-1 secretion in our experimental system.

NF-κB has been shown to regulate transcription of many pro-inflammatory cytokines and adhesion molecules and to be essential for MCP-1 gene expression in mesangial cells stimulated with interleukin-1β (Rovin et al., 1995; Stylianou et al., 1999). In resting cells, NF-κB is held inactive in the cytoplasm and forms a complex with IκB. Upon stimulation with interleukin-1β, IκB is proteolysed to release active NF-κB, which translocates to the nucleus, binds its target promoter and activates transcription (Barmes and Karin, 1997). Since tranilast inhibited NF-κB-driven luciferase activity stimulated by interleukin-1β, inhibition of MCP-1 expression by tranilast is likely to occur, at least partly, via suppression of NF-κB activation. However, electromobility shift assay demonstrated that tranilast did not inhibit interleukin-1β-induced DNA-binding activity of NF-κB. Similar findings have been recently reported in an abstract form that tranilast inhibits NF-κB-dependent transcriptional activation of endothelial cell adhesion molecules without affecting DNA-binding activity of NF-κB (Spiecker et al., 2000). In this context, recent evidence indicates that DNA binding of NF-κB is not sufficient for full activation of NF-κB (Bergman et al., 1998). Several steps have been suggested to regulate transactivation of NF-κB after DNA binding, including phosphorylation of NF-κB RelA/p65 subunit (Egan et al., 1999), recruitment of nuclear coactivator proteins (Gerritsen et al., 1997) and interaction between RelA/p65 and RNA polymerase II (Qiu et al., 1999). At which step(s) tranilast inhibits NF-κB activation after its DNA binding remains to be elucidated.

In addition to NF-κB, it is also possible to speculate that activities of other transcriptional factors be affected by tranilast. For instance, 12-*o*-tetradecanoylphorbol-13-acetate response element (TRE), which is activator protein-1 (AP-1) binding site, is also reported to be located on the 5′ flanking region of MCP-1 gene (Shyy et al., 1995). In our experiment, however, since interleukin-1β activated DNA binding of AP-1 only modestly in mesangial cells, the effect of tranilast on interleukin-1β-induced DNA binding of AP-1 was difficult to evaluate (data not shown).

Recent evidences have highlighted the involvement of MAP kinases in the activation of NF-κB. As for the JNK pathway, it has been demonstrated that dominant negative mutants of JNK inhibit the transcriptional activity of NF-κB in monocytes (Tuyt et al., 1999). The present study demonstrated that, of the three MAP kinases stimulated by interleukin-1β, tranilast suppresses JNK activation selectively, while activation of neither ERK nor p38 kinase is affected. In mesangial cells, recent studies demonstrated the JNK pathway to be necessary for interleukin-1β-induced cyclooxygenase and inducible nitric oxide synthase expression, both of which require NF-κB activation for gene transcription (Guan et al., 1998, 1999). Although the precise role of the JNK pathway in MCP-1 expression requires further investigation, these observations raise the possibility that suppression of the JNK pathway is involved in the inhibition of NF-κB activity by tranilast in mesangial cells.

Recent studies indicate that MCP-1 expressed in glomeruli plays a crucial role in the progression of glomerular injury by recruiting macrophages (Segerer et al., 2000). With regard to the cellular source of MCP-1 expression in glomeruli, several reports have suggested an important role of mesangial cells (Stahl et al., 1993; Gesualdo et al., 1997). Since interleukin-1β, a potent inducer of chemokine synthesis in mesangial cells, is also upregulated in the glomeruli during human and experimental glomerulonephritis (Matsumoto, 1990; Jenkins et al., 1994), it seems reasonable that inhibition of interleukin-1β-induced MCP-1 in mesangial cells be considered a valid means of blocking glomerular inflammatory responses. In this context, although glucocorticoids and immunosuppressant have been regarded as effective agents for human glomerulonephritis, various systemic side effects of these agents frequently cause problems in the clinical setting to date. On the other hand, in the TREAT study, the major adverse effect by tranilast administered at

doubled standard clinical dose (600 mg/day) was mild liver dysfunction, which occurred in less than 5% of subjects (Tamai et al., 1999). Therefore, tranilast may be highlighted as a safe oral agent that inhibits NF-κB activation and MCP-1 production in mesangial cells.

In conclusion, our results show that tranilast inhibits interleukin-1β-induced MCP-1 expression in mesangial cells, at least in part, by suppressing NF-κB activation. Selective inhibition of JNK activation among MAP kinases has the potential to contribute to the inhibitory effects of tranilast, although further studies are necessary to fully elucidate the mechanism of transcriptional inhibition. This study suggests that tranilast, as an anti-chemokine agent, may be useful for preventing glomerulonephritis.

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