TAK1 plays a major role in growth factor-induced phenotypic modulation of airway smooth muscle

Tonio Pera, Riham Sami, Johan Zaagsma, and Herman Meurs

Department of Molecular Pharmacology, University Centre for Pharmacy, University of Groningen, Groningen, The Netherlands

Submitted 18 January 2011; accepted in final form 22 August 2011

Pera T, Sami R, Zaagsma J, Meurs H. TAK1 plays a major role in growth factor-induced phenotypic modulation of airway smooth muscle. Am J Physiol Lung Cell Mol Physiol 301: L822–L828, 2011. First published August 26, 2011; doi:10.1152/ajplung.00017.2011.—Increased airway smooth muscle (ASM) mass is a major feature of asthma and chronic obstructive pulmonary disease. Growth factors induce a proliferative ASM phenotype, characterized by an increased proliferative state and a decreased contractile protein expression, reducing contractility of the muscle. Transforming growth factor-β-activated kinase 1 (TAK1), a mitogen-activated protein kinase kinase kinase, is a key enzyme in proinflammatory signaling in various cell types; however, its function in ASM is unknown. The aim of this study was to investigate the role of TAK1 in growth factor-induced phenotypic modulation of ASM. Using bovine tracheal smooth muscle (BTSM) strips and cells, as well as human tracheal smooth muscle cells, we investigated the role of TAK1 in growth factor-induced proliferation and hypocontractility. Platelet-derived growth factor- (PDGF; 10 ng/ml) and fetal bovine serum (5%)-induced increases in DNA synthesis and cell number in bovine and human cells were significantly inhibited by pretreatment with the specific TAK1 inhibitor LL-Z-1640-2 (SZ-7-oxozeaenol; 100 nM). PDGF-induced DNA synthesis and extracellular signal-regulated kinase-1/2 phosphorylation in BTSM cells were strongly inhibited by both LL-Z-1640-2 pretreatment and transfection of dominant-negative TAK1. In addition, LL-Z-1640-2 inhibited PDGF-induced reduction of BTSM contractility and smooth muscle α-actin expression. The data indicate that TAK1 plays a major role in growth factor-induced phenotypic modulation of ASM.

Airway smooth muscle (ASM) thickening is a pathological feature of asthma and chronic obstructive pulmonary disease (COPD), which may contribute to airflow limitation and airway hyperresponsiveness (36). The mechanisms underlying ASM remodeling have not been fully elucidated; however, there is evidence that ASM cell proliferation (9, 52), which can be induced by increased expression of growth factors in the airway wall (1, 2), may be involved. Growth factors induce a proliferative ASM phenotype, which is characterized by increased ASM cell proliferation and a decreased expression of contractile proteins, leading to decreased contractility (11, 13, 15, 16). Previous studies have shown that extracellular signal-regulated kinase (ERK)-1/2 is a key enzyme in platelet-derived growth factor (PDGF)-induced proliferation (11, 13, 23, 27) and decrease in contractility of ASM (11, 13). Indeed, induction of sustained ERK-1/2 phosphorylation is required for cell cycle progression and proliferation of ASM cells (24, 34). Transforming growth factor-β (TGF-β)-activated kinase 1 (TAK1), a serine/threonine kinase, is a member of the mitogen-activated protein (MAP) kinase kinase kinase (MAP3K) family (MAP3K7). Initially, TAK1 was identified as a mediator of TGF-β and bone morphogenetic protein signaling (53), but has since emerged as a key player in interleukin-1 (IL-1) receptor (30), toll-like receptor (18) and tumor necrosis factor-α (TNF-α) receptor (40) signaling. TAK1 activates both nuclear factor-κB (40, 41) and MAP kinase (53) pathways, including the ERK-1/2 pathway (8, 31, 45, 46). TAK1 has been found to play a major role in various immune responses (18, 42, 43, 51) and embryonal development (19, 20, 25, 32). In addition, accumulating evidence suggests that TAK1 plays a role in cardiac muscle hypertrophy (28, 54), indicating that this enzyme may also be involved in the pathogenesis of tissue remodeling. The role of TAK1 in ASM function is currently unknown. In this study, we present evidence that TAK1 plays a major role in growth factor-induced proliferation and reduced contractility of ASM.

MATERIALS AND METHODS

Isolation of tracheal smooth muscle cells. Bovine tracheae were obtained from local slaughterhouses and transported to the laboratory in Krebs-Henseleit (KH) buffer of the following composition (mM): 117.5 NaCl, 5.60 KCl, 1.18 MgSO4, 2.50 CaCl2, 1.28 NaH2PO4, 25.00 NaHCO3, and 5.50 glucose, pregassed with 5% CO2 and 95% O2; pH 7.4. After dissection of the smooth muscle layer and removal of mucosa and connective tissue, airway smooth muscle tissue was chopped using a McIlwain tissue chopper, three times at a setting of 500 μm and three times at a setting of 100 μm. Tissue particles were washed twice with Dulbecco’s modified Eagle’s medium (DMEM), supplemented with NaHCO3 (7 mM), HEPES (10 mM), sodium pyruvate (1 mM), nonessential amino acid mixture (1:100), gentamicin (45 μg/ml), penicillin (100 U/ml), streptomycin (100 μg/ml), amphotericin B (1.5 μg/ml), and fetal bovine serum (FBS, 0.5%) (all purchased from GIBCO BRL Life Technologies, Paisley, UK). Enzymatic digestion was performed using the same medium, supplemented with collagenase P (0.75 mg/ml, Boehringer, Mannheim, Germany), papain (1 mg/ml, Boehringer), and soybean trypsin inhibitor (1 mg/ml, Sigma Chemical, St. Louis, MO). During digestion, the suspension was incubated in an incubator shaker, three times at a setting of 500 μm and three times at a setting of 100 μm. Tissue particles were washed twice with Dulbecco’s modified Eagle’s medium (DMEM), supplemented with NaHCO3 (7 mM), HEPES (10 mM), sodium pyruvate (1 mM), nonessential amino acid mixture (1:100), gentamicin (45 μg/ml), penicillin (100 U/ml), streptomycin (100 μg/ml), amphotericin B (1.5 μg/ml), and fetal bovine serum (FBS, 0.5%) (all purchased from GIBCO BRL Life Technologies, Paisley, UK). Enzymatic digestion was performed using the same medium, supplemented with collagenase P (0.75 mg/ml, Boehringer, Mannheim, Germany), papain (1 mg/ml, Boehringer), and soybean trypsin inhibitor (1 mg/ml, Sigma Chemical, St. Louis, MO). During digestion, the suspension was incubated in an incubator shaker (Innova 4000) at 37°C, 55 rpm for 20 min, followed by a 10-min period of shaking at 70 rpm. After filtration of the obtained suspension over a 50-μm gauze, cells were washed three times in supplemented DMEM containing 10% FBS. This isolation method results in a cell population positive for smooth muscle α-actin and smooth muscle myosin heavy chain (11, 44).

Human tracheal sections from anonymized lung transplantation donors were obtained from the Department of Cardiothoracic Surgery, University Medical Centre Groningen, and transported to the laboratory in ice-cold KH buffer. Human tracheal smooth muscle (HTSM)
layer was prepared as described for bovine tracheal smooth muscle (BTSM) and chopped using a McIlwain tissue chopped. Tissue slices were washed once with supplemented DMEM, placed in culture flasks, and allowed to adhere. Upon reaching confluence, cells were passaged by trypsinization. Cells from passages 1–5 were used for the present study.

\[ \text{[H]} \text{thymidine incorporation. BTSM or primary HTSM cells were plated in 24-well cluster plates at a density of 30,000 cells per well and were allowed to attach overnight in 10% FBS-containing DMEM in a humidified 5% CO}_2 \text{ incubator at 37°C. Cells were washed twice with sterile phosphate-buffered saline [PBS, composition (mM) 140.0 NaCl; 2.6 KCl; 1.4 KH}_2\text{PO}_4; 8.1 Na}_3\text{HPO}_4; \text{pH 7.4] and made quiescent by incubation in FBS-free DMEM supplemented with apo-transferrin (5 µg/ml, human, Sigma), ascorbate (100 µM, Merck, Darmstadt, Germany), and insulin (1 µM, bovine pancreas, Sigma) for 72 h. Cells were then washed with PBS and stimulated with PDGF (10 ng/ml) or FBS (5%) in FBS-free DMEM for 28 h, in the presence of absence of the TAK1 inhibitor LL-Z1640-2 (100 nM, Bioaustalis, Darmstadt, Germany), and insulin (1 µM, bovine pancreas, Sigma) for 72 h. Cells were then washed with PBS and stimulated with PDGF (10 ng/ml) or FBS (5%) in FBS-free DMEM for 28 h, in the presence of absence of the TAK1 inhibitor LL-Z1640-2 (100 nM, Bioaustalis, Darmstadt, Germany), and insulin (1 µM, bovine pancreas, Sigma) for 72 h.}

\[ \text{Alamar blue assay. BTSM cells were plated, cultured, and made quiescent as described above. Cells were then stimulated with PDGF (10 ng/ml) or FBS (5%) for 4 days. After 4 days of stimulation, cells were washed twice with PBS and incubated with Hanks’ balanced salt solution containing 5% Alamar blue (BioSource, Camarillo, CA) solution for 45 min. Proliferation was assessed by conversion of Alamar blue, as indicated by the manufacturer.}

\[ \text{Transfection of plasmid DNA. For proliferation studies, cells were grown to 95% confluence on 24-well plates, washed twice with PBS, and then transfected using a mixture of 10 ng/lipofectamine 2000 and 0.4 µg DNA encoding a hemaglutinin-tagged, kinase-dead TAK1 mutant (TAK1 K63W) or green fluorescent protein (GFP), as control, with lipofectamine 2000 and lipofectamine 2000 (10 ng/ml, Amersham, Buckinghamshire, UK), after which the cells were washed twice with PBS and incubated with ice-cold 5% trichloroacetic acid on ice for 30 min. Subsequently, the acid-insoluble fraction was dissolved in 0.5 ml NaOH (1 M). Incorporated [H]thymidine was quantified by liquid-scintillation counting.}

\[ \text{Western blot analysis. BTSM cells were grown to confluence on six-well plates, washed twice with PBS, and then transfected using a mixture of 2 µl lipofectamine 2000 and 0.4 µg DNA encoding a hemaglutinin-tagged, kinase-dead TAK1 mutant (TAK1 K63W) or green fluorescent protein (GFP), as control, for 6 h in 200 µl DMEM without serum and antibiotics. After 6 h, cells were washed twice with PBS, the medium was changed to DMEM with antibiotics and insulin (1 µM), and the cells were then cultured for another 72 h.}

\[ \text{For Western blotting, cells were grown to 95% confluence on six-well plates and transfected using a mixture of 10 µl lipofectamine 2000 and 2 µg DNA encoding TAK1 K63W or GFP for 6 h in 600 µl DMEM without serum and antibiotics. After 6 h, cells were washed twice with PBS, the medium was changed to DMEM supplemented with antibiotics and 10% FBS, and the cells were then cultured for another 18 h. Dominant-negative (DN) TAK1 was a generous gift from Dr. B. J. L. Eggen (10) (Department of Developmental Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, The Netherlands), with kind permission of K. Matsumoto (Department of Molecular Biology, Graduate School of Science, Nagoya University, Japan).}

\[ \text{Western blot analysis. BTSM cells were grown to confluence on six-well cluster plates, using DMEM containing 10% FBS. Cells were then washed twice with sterile PBS and made quiescent by incubation in FBS-free DMEM, supplemented with insulin (1 µM), apo-transferrin (5 µg/ml), and ascorbate (100 µg/ml) for 24 h. Cells were then washed with PBS and stimulated with PDGF (10 ng/ml), for up to 2 h, in FBS-free medium. Subsequently, cells were washed once with ice-cold PBS and then lysed in ice-cold RIPA buffer (composition: 50 mM Tris, 150 mM NaCl, 1% Igepal CA-630, 1% deoxycholic acid, 1 mM NaF, 1 mM Na}_3\text{PO}_4, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 7 µg/ml pepstatin A, 5 mM 2-glycerophosphoric acid, pH 8.0). Lysates were stored at −80°C until further use. Protein content was determined according to Bradford (3). Total cell homogenates were then subjected to immunoblot analysis using antibodies against p-ERK-1/2 (Thr202/Tyr204), ERK-1/2 (Cell Signaling Technology), smooth muscle α-actin (Sigma), GAPDH (Santa Cruz Biotechnology) or hemaglutinin (Roche). The antibodies were visualized using enhanced chemiluminescence. Photographs of the blots were analyzed by densitometry.}

\[ \text{Tissue culture. After dissection of the smooth muscle layer and careful removal of mucosa and connective tissue, BTSM strips were prepared while incubated in gassed KH buffer at room temperature. Care was taken to cut tissue strips with macroscopically identical length (1 cm) and width (2 mm). Tissue strips were washed once in sterile FBS-free DMEM, supplemented with apo-transferrin (5 µg/ml) and ascorbate (100 µM). Next, the tissue strips were transferred into suspension culture flasks containing 7.5 ml FBS-free DMEM and were cultured for 4 days in the absence or presence of PDGF (10 ng/ml) and/or LL-Z1640-2 (100 nM).}

\[ \text{Isometric tension measurements. Tissue strips, collected from the suspension culture flasks, were washed with several volumes of KH buffer pregassed with 5% CO}_2 \text{ and 95% O}_2, \text{ pH 7.4 at 37°C. Subsequently, the strips were mounted for isometric recording (Grass force-displacement transducer FT03) in 20-ml water-jacked organ baths containing KH buffer at 37°C, continuously gassed with 5% CO}_2 \text{ and 95% O}_2, \text{ pH 7.4. During a 90-min equilibration period, with washouts every 30 min, resting tension was gradually adjusted to 3 g. Subsequently, the muscle strips were precontracted with 20 and 40 mM isotonic KCl solutions. Following two washouts, maximal relaxation was established by the addition of 0.1 µM (-)-isoprenaline (Sigma). In most of the experiments, no basal myogenic tone was detected. Tension was readjusted to 3 g, immediately followed by three washes with fresh KH buffer. After another equilibration period of 30 min, cumulative concentration response curves were constructed using stepwise increasing concentrations of isotonic KCl (5.6–50 mM) or methacholine (1 nM-100 µM; ICN Biomedicals, Costa Mesa, CA). When maximal tension was obtained, the strips were washed several times, and maximal relaxation was established using 10 µM (-)-isoprenaline.}

\[ \text{Data analysis. All data represent means ± SE from separate experiments. The statistical significance of differences between data was determined either by one-way ANOVA, followed by Bonferroni multiple-comparison test, or by the Student’s t-test, as appropriate. Differences were considered to be statistically significant when P < 0.05.}

\[ \text{RESULTS}

\[ \text{TAK1 regulates proliferation of BTSM and HTSM cells. To investigate the role of TAK1 in the proliferation of BTSM cells, [H]thymidine incorporation and Alamar blue assays were performed. Stimulation of these cells with PDGF (10 ng/ml) or FBS (5%) resulted in an increase in [H]thymidine incorporation (3.0- and 3.7-fold, respectively, after 28 h, Fig. 1A), as well as an increase in cell numbers (1.4- and 2.4-fold, respectively, after 4 days; Fig. 1B). Pretreatment of these cells with the specific TAK1 inhibitor, LL-Z-1640-2 (5Z-7-oxozeaenol; 100 nM) (8, 29) fully inhibited the PDGF-induced increase in [H]thymidine incorporation, whereas the FBS-induced response was inhibited by 69% (Fig. 1A). Similarly, LL-Z-1640-2 pretreatment abolished the PDGF-induced increase in cell number and inhibited the FBS-induced increase by 55% (Fig. 1B). As in BTSM cells, pretreatment with LL-Z-1640-2 abolished the PDGF-induced increase in [H]thymidine incorporation (Fig. 1C) and inhibited the FBS-induced increase in cell number by 62% (Fig. 1D) in primary HTSM cells. Transfection of BTSM cells with TAK1 DN resulted in a significant decrease of [H]thymidine incorporation, compared with GFP-transfected cells (Fig. 2). Collectively, these
data suggest that TAK1 plays a major role in growth factor-induced proliferation in both bovine and human ASM cells.

**TAK1 regulates ERK-1/2 phosphorylation in BTSM cells.** To investigate the role of TAK1 in growth factor-induced ERK-1/2 phosphorylation, Western blot analysis was performed. Stimulation of BTSM cells with PDGF for 5 min and 2 h resulted in a 6.4- and 3.7-fold increase in ERK-1/2 phosphorylation, respectively (Fig. 3). LL-Z-1640-2 pretreatment inhibited the PDGF-induced increase at 5 min and 2 h by 68 and 73%, respectively (Fig. 3). In addition, the ability of PDGF to induce ERK-1/2 phosphorylation was also evaluated in cells transfected with TAK1 DN or GFP, as a control. Stimulation of GFP-transfected cells with PDGF for 5 min and 2 h resulted in a 5.4- and 3.5-fold increase in ERK-1/2 phosphorylation, respectively. In TAK1 DN-transfected cells, the PDGF-induced ERK-1/2 phosphorylation was inhibited by 55% at 5 min and 85% at 2 h, compared with GFP-transfected cells (Fig. 4). Neither LL-Z-1640-2 pretreatment nor TAK1 DN expression had a significant effect on basal ERK-1/2 phosphorylation. These data indicate that TAK1 plays a major role in PDGF-induced ERK-1/2 phosphorylation.

**TAK1 regulates contractility of BTSM tissue preparations.** Previous studies have shown that growth factor-induced ASM cell proliferation strongly correlates with the capacity of growth factors to induce a decrease in contractility of ASM tissue preparations (11), which is associated with a decrease in contractile protein expression. To investigate the potential role of TAK1 in the development of growth factor-induced hypo-

---

**Fig. 1.** Growth factor-induced proliferation is inhibited by the transforming growth factor-β-activated kinase 1 (TAK1) inhibitor LL-Z-1640-2. A: [³H]thymidine incorporation in bovine tracheal smooth muscle (BTSM) cells. B: BTSM cell numbers determined by Alamar blue. C: [³H]thymidine incorporation in primary human tracheal smooth muscle (HTSM) cells. D: HTSM cell numbers determined by Alamar blue. Cells were stimulated with PDGF (10 ng/ml) or fetal bovine serum (FBS; 5%) in the presence or absence of LL-Z-1640-2 (100 nM). Values are expressed as percentage of basal controls and are means ± SE of 6–7 experiments, each performed in triplicate. *P < 0.05, ***P < 0.001 vs. untreated control. #P < 0.05 vs. treatment in the absence of inhibitor. NS, not significant vs. untreated control.

**Fig. 2.** PDGF-induced [³H]thymidine incorporation is inhibited by dominant-negative TAK1 (TAK1 DN). BTSM cells transfected with green fluorescent protein (GFP; control) or TAK1 DN were stimulated with PDGF (10 ng/ml). Data are expressed as fold increase from respective unstimulated, transfected cells and are means of 5–7 experiments, each performed in triplicate. *P < 0.05 vs. GFP.
contractile phenotype, we performed isometric contraction experiments using BTSM strip preparations. After 4 days of tissue culture in the presence of PDGF, there was a significant decrease in maximal contraction to both methacholine (Fig. 5A) and KCl (Fig. 5B). The decrease in contractility to both stimuli was strongly inhibited in the presence of LL-Z-1640-2 (Fig. 5A and B). Accordingly, LL-Z-1640-2 abolished the PDGF-induced decrease in smooth muscle α-actin expression in these tissue preparations. Collectively, our data indicate that TAK1 plays a major role in the induction of a proliferative, hypocontractile ASM phenotype by PDGF.

DISCUSSION

This is the first study addressing the role of TAK1 in ASM. In this study, we demonstrated that TAK1 is importantly involved in growth factor-induced ASM cell proliferation, ERK-1/2 phosphorylation, hypocontractility, and reduced contractile protein expression. Collectively, the data indicate that TAK1 plays a major role in the induction of a proliferative, hypocontractile ASM phenotype by PDGF.

Phenotypic modulation of ASM to a proliferative, hypocontractile phenotype occurs in response to a variety of mitogens, including growth factors (11, 13), G-protein-coupled receptor agonists (12, 26, 50), extracellular matrix proteins (6, 17), and, as recently shown, cigarette smoke and lipopolysaccharide (LPS) (35). The capacity of mitogens to induce ASM cell proliferation and decreased expression of contractile proteins has been found to correlate strongly with their capacity to decrease contractility of the ASM tissue preparations (6, 11). In addition, ERK-1/2 signaling induced by these mitogens has been shown to be fundamental to ASM cell proliferation (11, 13, 23, 24, 27, 34) and the associated decrease in ASM contractility (11, 13). Induction of a proliferative phenotype of ASM may cause ASM thickening (7, 14) and thus contribute to decline in lung function and airway hyperresponsiveness in chronic pulmonary diseases like asthma and COPD (36). Our present data, therefore, indicate that TAK1 could be importantly involved in the progression of asthma and COPD.

TAK1 has previously been shown to play a role in cardiac hypertrophy as well. TAK1 activity was increased in mouse myocardium after pressure overload (54), as well as in the non-infarcted cardiomyocytes, in a rat model of myocardial infarction (28). In both cases, this was associated with the subsequent development of cardiac hypertrophy. Indeed, the sole expression of activated TAK1 in the myocardium of transgenic mice was sufficient to induce cardiac hypertrophy (54). Interestingly, in addition to airway and cardiac remodeling, TAK1 has also been involved in embryonal development. Thus, in the mouse embryo, TAK1 is highly expressed in a variety of tissues (19). TAK1-deficient mouse embryos are not viable (20, 45) and show an abnormal, undeveloped vasculature, lacking vascular smooth muscle (20). Moreover, Tab1 gene mutant mouse embryos, which lack a functional TAK1-binding protein (TAB1) and TAK1 activity, also demonstrated abnormal development of the vasculature and the lung (25). Remarkably, a recent study in a Dutch birth

![Fig. 3. PDGF-induced ERK-1/2 phosphorylation is inhibited by LL-Z-1640-2. Top: BTSM cells were treated with PDGF (10 ng/ml) for 5 min or 2 h in presence or absence of LL-Z-1640-2. Cell lysates were analyzed by immunoblotting for p-ERK-1/2 and total ERK-1/2. Bottom: densitometry data. Data are normalized to the maximal response in each individual experiment and are means ± SE of 5 experiments. **P < 0.01, ***P < 0.001 vs. basal control. #P < 0.05, ###P < 0.001 vs. PDGF treatment in the absence of inhibitor.](http://ajplung.physiology.org/)

![Fig. 4. PDGF-induced ERK-1/2 phosphorylation (p) is inhibited by TAK1 DN protein expression. A: BTSM cells transfected with TAK1 DN or GFP were treated with PDGF for 5 min or 2 h. Cell lysates were analyzed by immunoblotting for p-ERK-1/2 and total ERK-1/2. B: expression of TAK1 DN was confirmed by immunoblotting for hemagluttinin (HA). Bottom: densitometry data are normalized to the maximal response in each individual experiment and are means ± SE of 7 experiments. **P < 0.01, ***P < 0.001 vs. untreated GFP-transfected control cells. #P < 0.05 vs. PDGF-treated, GFP-transfected control cells.](http://ajplung.physiology.org/)
cohort (38) has identified single-nucleotide polymorphisms located in the \( Tαb1 \) and \( Tαb2 \) genes, encoding TAK1-binding proteins, which regulate TAK1 activation, which were associated with asthma. Although the functional consequence of these single-nucleotide polymorphisms remains to be determined, this finding could implicate TAK1 signaling in the pathogenesis of asthma.

In addition to its inhibitory effect on ASM cell proliferation and ERK-1/2 phosphorylation, the TAK1 inhibitor LL-Z-1640-2 also inhibited the PDGF-induced decrease in maximal contraction to both a receptor-dependent stimulus, methacholine, and a receptor-independent stimulus, KCl. This suggests that PDGF induces a downstream effect and is confirmed by the decreased expression of the contractile protein, sm-\( α \)-actin. These data are consistent with the inhibition of a growth factor-induced shift to a proliferative, hypocontractile phenotype.

Our data identify TAK1 as a novel mediator of growth factor-induced proliferation of ASM cells. TAK1 has thus far not been implicated in PDGF-induced receptor tyrosine kinase signaling. In our study, PDGF-induced DNA synthesis and cell proliferation were fully inhibited, whereas the FBS-induced effects were only partially reduced by the TAK1 inhibitor LL-Z-1640-2. This observation suggests that mitogenic components of FBS may also increase cell proliferation via signaling pathways independent of TAK1. Although PDGF is one of the major mitogenic components of FBS, differences in pro-proliferative signaling induced by PDGF and FBS in ASM cells have been reported previously (13, 14, 47). For example, Rho kinase was shown to play a role in FBS-induced proliferation of ASM cells (47), whereas it was not involved in proliferation or hypocontractility induced by PDGF (13).

TAK1 has been shown to play a role in ERK-1/2 phosphorylation induced by bone morphogenetic protein in mouse chondrocytes (45, 46), IL-1β in mouse embryonal fibroblasts (45, 46), LPS and TNF-\( α \) in human neutrophils (8, 37), and LPS and phorbol-12-myristate-13-acetate in human peripheral blood monocytes (8, 37). Interestingly, in HeLa cells, TAK1 was shown to mediate TNF-\( α \)- but not EGF-induced ERK-1/2 phosphorylation (31), indicating that the contribution of TAK1 to ERK-1/2 signaling may be stimulus specific. Coimmunoprecipitation analysis has been used to show inducible interaction of TAK1 with Ras in caveolae of rat hepatic macrophages (5). TAK1 has also been found to mediate resistance to apoptosis in H-Ras-transformed human bladder carcinoma T24

Fig. 5. PDGF-induced decrease in contractility and contractile protein expression in BTSM tissue are inhibited by LL-Z-1640-2. Methacholine- (MCh) (A) and KCl (B)-induced contractions of BTSM strips cultured for 4 days in the absence or presence of PDGF (10 ng/ml), with or without LL-Z-1640-2 (100 nM), are shown. C: contractile protein expression in cultured BTSM strips was determined by immunoblotting for \( α \)-actin and GAPDH as loading control. \( α \)-Actin blots were analyzed by densitometry. A representative blot is shown. Values are means ± SE of 4 – 6 experiments. \(* P < 0.05 \) vs. basal control. \#P < 0.05 vs. PDGF. NS, not significant vs. basal control.
Peripheral monocytes with an IC50 range (10–25 nM) similar to IL-1, IL-6 production by human H9251/H9252 (31) or HeLa cells (48), indicating that the inhibitor is specific to the values observed in the in vitro kinase assay mentioned above. Moreover, pretreatment of mice with LL-Z-1640-2 (intraperitoneal injection; 3–30 mg/kg body mass) dose-dependently inhibited the LPS-induced increase of serum TNF-\(\alpha\) concentration, indicating the potential for use of this inhibitor in vivo (37). Importantly, in the present study, the specificity of LL-Z-1640-2 was confirmed by the inhibition of PDGF-induced ERK-1/2 phosphorylation in cells transfected with TAK1 DN.

In conclusion, our study has identified TAK1 as a novel regulator of growth factor-induced proliferation in ASM cells. TAK1 may, therefore, regulate phenotypic modulation of ASM cells and could contribute to the development of ASM remodeling in obstructive airways diseases.

ACKNOWLEDGMENTS

The authors thank Dr. B. G. J. Dekkers for technical assistance.

GRANTS

This study was financially supported by Boehringer Ingelheim Pharma and the Graduate School of Behavioral and Cognitive Neurosciences, University of Groningen, The Netherlands.

DISCLOSURES

No conflicts of interest, financial or otherwise are declared by the author(s).

REFERENCES

TAKE 1 REGULATES AIRWAY SMOOTH MUSCLE PHENOTYPE


