New anti-fibrotic mechanisms of n-acetyl-seryl-aspartyl-lysyl-proline in silicon dioxide-induced silicosis

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Aims: We previously reported that tetrapeptide N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP) inhibited pulmonary inflammation and fibrosis in SiO2-induced silicosis. This study aimed to explore the precise mechanism involved.

Main methods: Rats were divided into 3 groups: 1) sham (saline), 2) silicosis+ vehicle, and 3) silicosis+Ac-SDKP [800 μg/(kg·d)]. SiO2 particles or saline were administered by tracheal instillation and Ac-SDKP or vehicle (saline) via a miniosmotic pump planted into the abdominal cavity 48 h before instillation. Animals were observed for 4 weeks. Silicotic nodule fraction (SNF) and macrophage infiltration (ED-1 positive cells) were measured by hematoxylin and eosin (H.E.) and immunohistochemical staining respectively. Collagen I and III, transforming growth factor-β1 (TGF-β1) proteins and monocyte chemotactic protein-1 (MCP-1) mRNA were detected by Western Blot (WB) and real-time RT-PCR respectively. In vitro, pulmonary fibroblasts were stimulated by TGF-β1 (5 μg/ml) with or without Ac-SDKP. Phosphorylated c-Jun N-terminal Kinase (p-JNK) was detected by WB and p-JNK nuclear translocation by confocal analysis.

Key findings: SiO2 significantly increased the SNF, collagen I and III proteins, TGF-β1, MCP-1 mRNA and macrophage infiltration. All these pathological changes were inhibited by Ac-SDKP. TGF-β1 resulted in fibroblast proliferation, increased expression of collagen I and III proteins, p-JNK and its subsequent nuclear translocation. Addition of Ac-SDKP markedly suppressed these changes.

Significance: These data indicate that the anti-fibrotic effect of Ac-SDKP in silicosis is mediated by inhibiting chronic inflammation, TGF-β1 production, and TGF-β1-induced pulmonary fibroblast proliferation and collagen synthesis.

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Introduction

Silicosis is induced by inhalation of silica dioxide (SiO2) containing dusts into the lungs and is characterized by persistent inflammation, fibroblast proliferation and excess collagen deposition, ultimately resulting in interstitial fibrosis and formation of silicotic nodules (Thakur et al. 2009).

During development of silicosis, chronic inflammation mediated by alveolar macrophages is considered to be an initiating step. After deposition, SiO2 is engulfed by the macrophages. However, silica is toxic to the macrophages, leading to cell damage, death and liberation of free silica which is subsequently taken up by other macrophages. This recurring cycle of macrophage phagocytosis perpetuates the inflammatory process (Balaan and Banks 1998; Binaya et al. 2005). Furthermore, those live macrophages are being activated to release many inflammatory mediators which will intensify the chronic inflammation (Binaya et al. 2005).

Activated macrophages also secrete cytokines to promote pulmonary fibroblasts to proliferate and synthesize excess collagen, which directly contributes to formation of silicotic nodules and interstitial fibrosis (Zhai et al. 2004; Wang et al. 2009a,b). Among them, transforming growth factor-β (TGF-β) appears to be important. It has been reported that macrophages stimulated by quartz dust are able to synthesize TGF-β1 which is responsible for collagen synthesis of human lung fibroblasts and proliferation (Olbрук et al. 1998; Ji et al. 2009). By contrast, inactivation of TGF-β1 by silencing expression of CD36 in macrophages significantly reduces collagen production and prevents the development of silica-induced lung fibrosis (Wang et al. 2009a,b). TGF-β1 signaling from the cell membrane to the nucleus is mediated by intracellular effector molecules (Kanasaki et al. 2003), and recent evidence has shown that c-Jun N-terminal kinase (JNK) is a critical mediator in this downstream pathway. After TGF-β1 binding to its receptors, phosphorylated receptors, associated with TRAF6 and TAK1 (TGF-β-
activated kinase1), activates MKK4 (MAP kinase kinases 4), resulting in JNK phosphorylation and translocation to the nucleus. In the nucleus, JNK induces c-Jun protein phosphorylation and regulates the expression of a target gene (Zhang 2009).

Thus, prevention of development of inflammation and fibrosis is significant to improve the treatment. N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP) is an endogenous peptide released from its precursor (thymosin-4) by proline oligopeptidase normally found in human plasma and circulating monocytes (Guigon et al. 1990; Pradelles et al. 1991; Azizi et al. 1997; Cingolani et al. 2004). Ac-SDKP was originally described as a natural inhibitor of pluripotent hematopoietic stem cell proliferation (Lenfant et al. 1989). Now it is reported that a significant reduction of endogenous Ac-SDKP with a specific prolyl oligopeptidase inhibitor in normal rats promotes heart collagen deposition, perivascular fibrosis and glomerulosclerosis, suggesting that this peptide has a physiological role in preventing organ collagen accumulation (Maria et al. 2007). Furthermore, Ac-SDKP significantly prevents interstitial collagen deposition in hypertension and myocardial infarction (Peng et al. 2003; Yang et al. 2004; Lin et al. 2008).

However, there are few reports on the effect of Ac-SDKP on development of silicosis. We have previously shown that Ac-SDKP could reduce the size of silicotic nodules and collagen deposition, which is associated with the decrease in macrophage infiltration in SiO₂-treated rats, indicating that Ac-SDKP has an inhibitory effect on the SiO₂-induced fibrosis and this effect is partially mediated by inhibition of inflammation (Li et al. 2008; Yan et al. 2008). As the important role of fibroblast proliferation and collagen synthesis in silicotic fibrosis, it is essential to further investigate the interaction between Ac-SDKP and them as well as the involved mechanisms. Here, we hypothesized that Ac-SDKP, mediated by the modulation of TGF-β1 expression and TGF-β1/JNK signaling pathway, inhibits pulmonary fibroblast proliferation and collagen synthesis.

Materials and methods

Animals

Male Wistar rats, weighting 180 g, were purchased from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). Rats were housed in an air-conditioned room with a 12-h light/dark cycle and given standard chow and tap water. Animals were given 1 week to adjust to their new environment before the experiment. All studies in animals were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Induction of silicosis and Ac-SDKP treatment

Crystalline SiO₂ (approx. 80% between 1 and 5 μm), obtained from Sigma (St. Louis, MO, USA), was acid washed, dried and autoclaved. Rats were divided into 3 groups (n = 10): 1) sham (saline), 2) silicosis + vehicle, and 3) silicosis + Ac-SDKP. Sham or silicosis were created by instilling normal saline or SiO₂ (50 mg) suspended in 1 ml of normal saline through the trachea. Ac-SDKP [800 μg/(kg d), Bachem AG company, USA] or vehicle (saline) was given via a miniosmotic pump planted into the abdominal cavity 48 h before induction of silicosis. The animals were observed for 4 weeks.

Histopathology

At the end of the study, rats were sacrificed and the lungs were removed. To measure the size of silicotic nodules, the lower lobe of the right lung was fixed in formalin and embedded in paraffin. Six micron tissue sections were stained with Hematoxylin and Eosin (H.E.). Images in each rat were obtained from 5 separate randomly selected fields (200× magnification). Area of total surface (microscopic field) and of the silicotic nodules was measured with computer-assisted videodensitometry (Beijing University of Aeronautics and Astronautics, China). Silicotic nodules fraction was calculated by the percent total surface area occupied by silicotic nodules.

Hydroxyproline assay

Collagen content of the middle lobe of the right lung tissue was determined by hydroxyproline assay according to the instructions described (Nanjing Jiancheng Biological Engineering Co., Ltd, Jiangsu Province, China). Briefly, the tissue was lysed in lysis buffer and boiled for 20 min. After adjusting the pH to 6.0, activated carbon was added followed by centrifugation. The supernatant was collected and hydrolyzed with 6 N HCl for 15 min at 60 °C. The mixture was centrifuged and the hydroxyproline content of the supernatant was quantified by spectrophotometry at 550 nm. The data were expressed as collagen (μg)/dry weight (mg).

Western blot analysis of collagen type I, III and TGF-β1 levels

Upper lobe of right lung or cultured fibroblasts were lysed with lysis buffer, and then centrifuged at 14,000 g for 10 min. The supernatant was collected and protein content determined with a protein assay kit (Bio-Rad). The proteins (90 μg/lane) were separated in 8% gel by SDS-PAGE and electrotransferred to a nitrocellulose membrane (Amervehicle Biosciences). Membranes were blocked with 5% non-fat milk and incubated overnight at 4 °C with the primary antibody (anti-collagen type I or III, Sigma; Anti-TGF-β1, R&D system; anti-β-actin, Santa Cruz Biotechnology) followed by horse-radish peroxidase-conjugated secondary antibodies (Amervehicle Biosciences). Target bands were visualized by addition of diaminobenzidine (DAB). Results were expressed as the ratio of the density of specific bands to the corresponding β-actin band.

Real-Time quantitative RT-PCR to detect MCP-1 mRNA expression (Choi et al. 2009)

Total RNA was extracted from the frozen tissue using Trizol Reagent (Invitrogen, USA). After 1 μg of total RNA was reverse transcribed into cDNA, real-time PCR was performed using SYBR Green PCR mastermix (Qiagen). The primers were synthesized by Shenggong Biotech Company Limited (Shanghai, China) and the sequences of MCP-1 primers were: 5′-ATGGCTCTGTGTCACG-3′ (sense) and 5′-ATGGTTCTCTGTCATACT-3′ (antisense). The sequences of GAPDH primers were: 5′-TCCAGCCCCATTAAACAG-3′ (sense) and 5′-TGAGGCTGCACGGACTTTA-3′ (antisense). PCR was performed using a thermal cycler (Rotor-Gene 3000, Corbett Research) and the conditions were as follows: denaturation at 95 °C for 10 min., followed by 40 cycles of 10 s at 95 °C, 15 s at 60 °C and 20 s at 72 °C. Each sample was analyzed in triplicate and normalized to GAPDH. The level of MCP-1 mRNA was expressed by the fold changes relative to the sham group.

Immunohistochemistry for macrophage infiltration

Paraffin-embedded sections were deparaffinized, rehydrated and boiled in a microwave oven for 10 min. After incubation with 5% horse serum, sections were incubated with primary antibodies against ED-1 (a marker for macrophages, 1:100, Lab Vision) followed by the biotinylated secondary antibody and finally the ABC reagent (Wuhan Boshide Biological Engineering Co., Ltd, China). Immunoreactivity was visualized with DAB. A brown color staining was considered a positive result. Sections were counterstained with hematoxylin. Images were obtained from 5 separate randomly selected fields (400× magnification). Quantitative analysis was performed blindly and the cells expressing ED-1 were counted in the entire image and expressed as number/mm².

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Fibroblast proliferation and collagen synthesis in vitro

Fibroblasts were isolated from the lungs 2–30 days old Wistar rats according to a previously described procedure (Li et al. 2008; Yan et al. 2008). Cells were cultured in 0.5% serum-DMEM and most cells were in quiescent state at passage 4. When cells reached 70% confluence, they were divided into 3 groups: 1) control (0.5% serum-DMEM); 2) TGF-β1 (5 μg/ml, R&D) + vehicle; and 3) TGF-β1+ Ac-SDKP. Ac-SDKP was added to the medium 30 min before TGF-β1 treatment. Cells were cultured for 48 h and their proliferation and collagen synthesis were determined using the MTT assay and Western blotting respectively.

Confocal microscopic analysis of phosphorylated-JNK nuclear translocation

Fibroblasts were grown and divided into 3 groups as described above. Ac-SDKP was added 30 min before TGF-β1 administration, and then continued to culture for 40 min. After washing, cells were fixed with 3% formaldehyde in PBS for 15 min at room temperature and then incubated with Triton X-100 (2% in PBS) for another 15 min. Cells were blocked with 1% bovine serum albumin in PBS for 1 h. They were then incubated for 1 h with to rabbit anti-phosphorylated-JNK (p-JNK) antibodies (1:200 dilution) followed by FITC-conjugated goat anti-rabbit antibody (Sigma) (1:100 dilution) for 1 h in the darkness. Cells were washed with PBS and then incubated with 4′,6-diamidino-2-phenylindole (DAPI) for counterstaining the nucleus. Slides were mounted in Vectashield fluorescence mounting media (Vector labs, Burlingame, CA) and visualized on a multichannel confocal laser-scanning microscope (Olympus FluoView 1000 LSCM; LUMPlanFl×40 W water-immersion objective. FITC was excited at 488 nm and DAPI at 405 nm. The fluorescence intensity was analyzed by the imaging analysis software (SV10-ASW; Olympus). p-JNK nucleus translocation was indicated by the ratio of green fluorescence intensity in the nucleus and cytoplasm.

Data analysis

All data are expressed as mean±SEM. Multiple group comparisons were conducted using ANOVA and Student’s t-test for pair-wise comparisons. Group differences resulting in p-values of less than 0.05 were considered to be statistically significant.

Results

The development of silicotic nodules and fibrosis

The important characters are inflammation, silicotic nodules and interstitial fibrosis in silicosis. Using rats with SiO2-induced silicosis, we firstly investigate the ability of Ac-SDKP to influence development of silicosis by H.E. staining. In the normal controls, the lungs showed thin alveolar septum without significant inflammation and remained unchanged during the study (Fig. 1). Four weeks of SiO2 stimulation caused significant changes in the lungs, characterized by chronic inflammation, widening of the alveolar septum and the development of silicotic nodules composed of macrophages and fibroblasts. In contrast, Ac-SDKP treatment significantly reduced the alveolar septum and decreased the size of nodules.

Pulmonary collagen deposition

As silicotic nodules and interstitial fibrosis are mainly composed of collagen types I and III, we detect the effect of Ac-SDKP on expression of collagen type I and III by hydroxyproline assay and western blot. There was little collagen types I and III in the lungs of the sham group (Fig. 2). As expected, four weeks of SiO2 stimulation markedly increased the collagen content and expression of both collagens I and III, while Ac-SDKP treatment significantly reduced these deposition.

Fig. 1. Histology and quantitative data on silicotic nodules in rats with sham silicosis or silicosis treated with vehicle or Ac-SDKP. (A): sham silicosis; (B): silicosis + vehicle; (C): silicosis + Ac-SDKP. The silicotic nodules are indicated by arrows. (D): quantitative data of silicotic nodular fraction (*p<0.05 vs sham, # p<0.05 vs vehicle).
Macrophages play an important role during the inflammatory response, and activated macrophages aggravate inflammation by releasing inflammatory mediators to attract more macrophages to foci. Monocyte chemoattractant protein-1 (MCP-1), mainly derived from macrophages and alveolar epithelial cells, is able to regulate migration and infiltration of macrophages (Deshmank et al. 2009). To investigate the effect of Ac-SDKP on chronic inflammation, we detect whether Ac-SDKP can inhibit macrophage infiltration and whether the inhibitory effects are mediated by suppressing MCP-1 expression. MCP-1 protein expression was detected by real-time RT-PCR and the infiltrating macrophages were quantitated with ED-1 (a marker for macrophage) immunohistochemical staining. As shown in Fig. 3, SiO₂ treatment resulted in a 3.8 fold increase in the number of macrophages (Fig. 4B and D), which was markedly prevented by Ac-SDKP (Fig. 4C and D).

**MCP-1 expression and macrophage infiltration in lung**

Activated macrophages also secrete cytokines to stimulate fibroblast proliferation and synthesize collagen, excess collagen deposition finally resulting in silicotic fibrosis. The best characterized of these cytokines is TGF-β1 (Olbrück et al. 1998; Wang et al. 2009a, b). In order to determine whether Ac-SDKP can suppress TGF-β1 production in SiO₂-induced rats, we test the level of TGF-β1 protein by western blot. As shown in Fig. 5, TGF-β1 expression in the lung was low in the sham group. SiO₂ markedly stimulated TGF-β1 production in the lungs, which was significantly reduced by Ac-SDKP treatment (Fig. 5).

**Fibroblast proliferation and collagen synthesis induced by TGF-β1 in vitro**

Besides reduction of TGF-β1 expression in silicosis, the further study is to investigate the possibility that Ac-SDKP is able to inhibit TGF-β1-stimulated fibroblast proliferation and collagen synthesis. Using cultured pulmonary fibroblasts, TGF-β1 effectively stimulates fibroblasts to proliferate (Fig. 6A). This effect can be partially abrogated by Ac-SDKP in a dose-dependent manner. The synthesis of collagens I and III are also induced by TGF-β1 (Fig. 6B). This effect can be partially abrogated by Ac-SDKP.

**Discussion**

Silicosis is a worldwide health issue especially in developing countries. For example, there are up to 10,000 new cases and 24,000
deaths every year in China (WHO Health Organization 2000). Silicosis is a progressive pulmonary fibrotic disease and is related to the development of lung cancer, which has no successful treatment available to date (Peretz et al. 2006). Ac-SDKP has been documented to have anti-inflammatory and anti-fibrotic properties when administered chronically in cardiovascular diseases, such as hypertension and myocardial infarction (Peng et al. 2003; Yang et al. 2004; Lin et al. 2008). Recently, we reported that this peptide exerts the same effects on SiO\textsubscript{2}-induced pulmonary silicosis in rats (Li et al. 2008; Yan et al. 2008). In the present study, we examined the precise mechanisms and found that Ac-SDKP prevents the development of pulmonary fibrosis and inflammatory process in vivo, through the inhibition of alveolar macrophage infiltration and decrease in TGF-β1. In addition, it also could suppress TGF-β1-stimulated pulmonary fibroblasts to proliferate and synthesize collagen by blocking JNK phosphorylation and nuclear translocation.

The cause of silicosis is mainly due to the inhalation of silica-containing dusts, which is very common to individuals employed in the mining and construction industries (Yucesoy et al. 2001). The most striking features are the presence of silicotic nodules and interstitial fibrosis. In experimental silicosis, granulomas (premature silicotic nodules, consisting mainly of macrophages and some fibrillar collagen) are formed within 1–2 weeks after silica administration (Reiser et al. 1982). They gradually developed into mature silicotic nodules associating with increased number of fibroblasts and collagen synthesis (Reiser et al. 1983). Consistent with “classic” silicosis, we found histologically there was prominent granuloma formation in lungs 4 weeks after SiO\textsubscript{2} instillation. They also had thicker alveolar walls, which were mainly composed of macrophages and fibroblasts. The main function of fibroblasts is to synthesize and secrete collagen proteins, while excess collagen deposition results in silicotic fibrosis. In this study, the collagen content and expression of type I and III collagen were significantly higher in the SiO\textsubscript{2}-treated group. However, these changes were significantly inhibited by chronic administration of Ac-SDKP. These observations suggest that exogenous administration of Ac-SDKP can prevent development of SiO\textsubscript{2}-induced fibrosis in vivo.

Although the mechanism of silicosis is not fully understood, chronic inflammation mediated by alveolar macrophages initiates development of silicosis (Binaya et al. 2005). Therefore, this study examined the effect of Ac-SDKP on macrophage infiltration in rats with SiO\textsubscript{2}-induced silicosis. We found that chronic treatment of Ac-SDKP significantly decreased the numbers of macrophages in the lungs as evidenced by the markedly decreased ED-1 positive cells in the Ac-SDKP group compared with the silicosis group. Similarly to our data, Lin et al. (2008) had found that in Angiotensin II-induced hypertension, the increased macrophages in the aortic wall were significantly attenuated by the Ac-SDKP treatment. Galectin-3, an indicator of macrophage activation, had been reported to be increased.

![Fig. 4. Immunohistochemical staining and quantitative data showing macrophage infiltration in rats. (A): sham silicosis; (B): silicosis + vehicle; (C): silicosis + Ac-SDKP; (D): number of ED-1 positive macrophages per unit area. Positive brown staining is located in the interstitial and alveolar space. *p<0.05 vs sham; # p<0.05 vs vehicle.](image)

![Fig. 5. Effect of Ac-SDKP on pulmonary TGF-β1 expression in rats. TGF-β1 was detected by Western blotting on lung tissue from rats with sham silicosis or silicosis treated with vehicle or Ac-SDKP. Its levels were determined by densitometry and normalized using β-actin. *p<0.05 vs sham; # p<0.05 vs vehicle.](image)
in the heart of animals under Angiotensin II induction. Ac-SDKP significantly reduced this change, suggesting that it could inhibit the infiltration of activated macrophages (Sharma et al. 2008). Macrophage infiltration is mainly controlled by MCP-1 which is a potent chemotactic factor (Ajuebor et al. 1998). MCP-1 binds to specific cell surface transmembrane receptors, whose activation leads to the activation of intracellular signaling cascades that prompt macrophage migration into foci (Callewaere et al. 2007). To explore the precious mechanisms by which Ac-SDKP inhibits macrophage infiltration, we detected the levels of MCP-1 mRNA and found the increased MCP-1 mRNA expression in silicosis could be significantly lessened by Ac-SDKP. Thus, it is proposed that the anti-macrophage infiltration property of Ac-SDKP in silicosis was mediated by inhibition of MCP-1 expression.

TGF-β is a multifunctional cytokine that regulates development, cell proliferation and matrix protein synthesis (Border and Noble 1994; Miyazono 2000; Kanasaki et al. 2003), and it is functional in stimulation of fibroblast proliferation and collagen synthesis in silicosis (Pittel et al. 2001; Daniel et al. 2003). Olbrück et al. had reported that the conditioned medium from human macrophages contained TGF-β1 (Olbrück et al. 1998). Using a quartz-induced silicosis model, Chen et al. also found that TGF-β1 expression was increased in the lung (Chen et al. 2005). Consistent with their results, we found that 4 weeks after SiO2 instillation, the pulmonary TGF-β1 expression increased nearly 3.6 folds, which could be markedly inhibited by chronic administration of Ac-SDKP. However, whether this property of Ac-SDKP on TGF-β1 expression was mediated either by inhibition of macrophage activation or TGF-β1 synthesis/secretion by activated macrophages needs further investigation. We also investigate whether Ac-SDKP directly suppresses the TGF-β1-induced pulmonary fibroblast proliferation and collagen synthesis in vitro. We used TGF-β1 to stimulate pulmonary fibroblasts and found that it significantly stimulated not only fibroblast proliferation but also synthesis of collagen I and III, and Ac-SDKP prevented these changes. Our data is in agreement with a previous report that Ac-SDKP can inhibit the TGF-β1-induced collagen and plasminogen activator inhibitor-1 (an extracellular matrix protease inhibitor) mRNA expression in mesangial cells via inhibition of Smad2 phosphorylation (Kanasaki et al. 2003). Taken together with our data regarding macrophage infiltration, the present study suggests that Ac-SDKP...
attenuates SiO2-induced fibrosis, partially by inhibiting chronic inflammation, TGF-β1 production and TGF-β1-induced pulmonary fibroblast proliferation and collagen synthesis.

After binding to its receptors, TGF-β1 stimulates intracellular JNK phosphorylation, and activated TGF-β1/JNK signaling pathway is implicated in fibroblast proliferation and collagen synthesis. Khalil et al. reported that TGF-β1-stimulated pulmonary interstitial fibroblast proliferation is mediated by the release of extracellular fibroblast growth factor-2 (FGF-2) act via the upregulation of phosphorylated-JNK (Khalil et al. 2005). He et al. illustrated that TGF-β1-induced Smad2/3/4 complex formation and nuclear translocation could promote keloid fibroblast proliferation and collagen deposition mediated by JNK, since inhibitors of JNK interrupted Smad2/3/4 complex translocation into the nucleus (He et al. 2009). In this study, we also focused on the TGF-β1/JNK signaling pathway to address possible mechanisms by which Ac-SDKP prevents TGF-β1-induced fibroblast proliferation and collagen synthesis. We found the expression of p-JNK was significantly increased in pulmonary fibroblasts cultured with TGF-β1, which is significantly inhibited by Ac-SDKP. It had been shown that p-JNK could move to the nucleus to carry out important cellular functions, such as alterations in gene expression (c-jun, c-fos, p53 and c-myc), apoptosis or cellular proliferation (Gupta et al. 1996; Adler et al. 1997; Noguchi et al. 1999; Bogoyevitch and Kobe 2006). We observed the translocation of p-JNK from the cytoplasm into the nucleus after TGF-β1 stimulation and this could be suppressed by Ac-SDKP. Taken together, our data suggest that Ac-SDKP inhibits TGF-β1-stimulated pulmonary fibroblast proliferation and collagen synthesis via prevention of JNK phosphorylation as well as p-JNK nuclear translocation.

However, how Ac-SDKP transduced the signal into the cytoplasm of macrophages and fibroblasts is unknown. Zhuo et al. (Zhuo et al. 2007) had reported that there is a single class of high-affinity receptor binding sites (Hpp-Aca-SDKP) in cultured cardiac fibroblasts which is a functional ligand for Ac-SDKP. Whether there are similar Ac-SDKP binding sites on pulmonary macrophages and fibroblasts in silicosis, which may lead to anti-fibrotic signal transduction needs to be clarified.

In summary, Ac-SDKP can inhibit pulmonary fibrosis in rats with SiO2-induced silicosis. This effect is mediated by inhibiting chronic inflammation, TGF-β1 production, and TGF-β1-induced pulmonary fibroblast proliferation and collagen synthesis. These findings suggest possible mechanisms by which pulmonary fibrosis develops in silicosis. More importantly, Ac-SDKP or its analogs may be useful in treating fibrosis in silicosis.

Conclusions

Ac-SDKP exhibited a protective effect against SiO2-induced silicosis in the lung, and this effect was mediated by inhibiting chronic inflammation, TGF-β1 production, and TGF-β1-induced pulmonary fibroblast proliferation and collagen synthesis. The findings suggest possible mechanisms by which pulmonary fibrosis develops in silicosis. More importantly, Ac-SDKP or its analogs may be useful in treating fibrosis in silicosis.

Fig. 7. Effect of Ac-SDKP on TGF-β1-stimulated JNK phosphorylation and nuclear translocation in pulmonary fibroblasts. (A) Representative Western blots (top) and corresponding densitometry data (bottom) showing the effect of Ac-SDKP on TGF-β1-stimulated p-JNK levels. The p-JNK levels have been normalized with total JNK. (B) Representative confocal images (top) and corresponding data (bottom, ratio of p-JNK in nucleus to cytoplasm) illustrating the effect of Ac-SDKP on p-JNK nuclear translocation. *p<0.05 vs control; #p<0.05 vs vehicle.
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Conflict of interest statement
The authors declare that there are no conflicts of interest.

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