J Med Dent Sci 2007; 54:

Original Article

TGF-β and glutathione promote tissue repair in cigarette smoke induced injury.

Chika Noguchi, Takeshi Umino, Yasunari Miyazaki, Torahiko Jinta, Yutaka Usui and Yasuyuki Yoshizawa

Department of integrated pulmonology, Tokyo Medical and Dental University, Tokyo, Japan

We studied the effect of cigarette smoke extract (CSE) on a three-dimensional (3-D) co-culture model with epithelial cells and mesenchymal cells to clarify how epithelial cells protect lung tissue from cigarette smoke *in-vivo*.

Two types of gels were prepared. The one was the co-culture of human fetal lung fibroblasts (HFL-I) embedded in type-I collagen gel, with alveolar epithelial cells (A549) cultured covering the top of the gel. The other was HFL-I cells alone. After 48 hours from CSE exposure, gel contraction, levels of fibronectin, transforming growth factor (TGF)-β1 and GSH were assessed.

CSE inhibited fibroblast-mediated gel contraction and this inhibition was lessened in co-culture associated with higher GSH concentration and TGF-β1 level as compared to the level in HFL-I cells alone. CSE lowered fibronectin level to a lesser extent in co-culture as compared to the level in HFL-I cells alone. Exogenous TGF-β1 restored the inhibition of gel contraction by CSE independent of GSH level.

Cigarette smoke may interfere with 3-D co-culture gel contraction by

diminishing GSH, fibronectin and TGF-β1 action in the epithelial-mesenchymal interaction.

Key words: chronic obstructive pulmonary disease, fibronectin, gel contraction, glutathione, transforming growth factor β

Corresponding Author: Yasuyuki Yoshizawa
Department of Integrated pulmonology, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo, 113-8519, Japan
e-mail: yoshizawa.pulm@tmd.ac.jp
Fax number: 81(0) 3 5803 0260
Received November 14; Accepted December 1, 2006

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by progressive destruction and permanent enlargement of peripheral airspaces of the lung. The greatest risk factor for COPD is cigarette smoking. The imbalance of protease/anti-protease and oxidants/anti-oxidants has been assumed to be the major pathogenic mechanisms for alveolar destruction. These processes have been considered to be regulated mainly by inflammatory cells such as neutrophils and macrophages[1]. However, these theories could not completely explain the pathogenesis of COPD. Thus alternative or additional mechanisms have recently emerged. Apoptosis of epithelial cells followed by alveolar wall degeneration is the one possible explanation and this process is independent of inflammatory cells[2]. Vascular atrophy caused by VEGF deficiency is also reported as a possible mechanism of alveolar destruction[3,4]. On the other hand, continuous repair processes following alveolar injury must be also important in the pathogenesis of COPD[5]. In the current study, we have focused on the effect of cigarette smoke on mesenchymal-epithelial interactions.

We designed an in-vitro three-dimensional (3-D) co-culture model that simulates alveolar walls. This culture system enables cross-talking between epithelial cells and mesenchymal cells through extracellular matrices without any interference of inflammatory cells such as macrophages and neutrophils. Mesenchymal-epithelial interactions are considered to be pivotal for epithelial homeostasis and regeneration. Local homeostasis is maintained either by resident cell response to external stimuli including interleukins derived from the bloodstream or by the cross-talk of growth factors such as transforming growth factor

(TGF)-β produced by resident cells *in vivo*. The interaction between epithelial cells and mesenchymal cells are thought to take place through their cell surface receptors and signal transduction.

The ability of fibroblasts to induce collagen gel contraction is thought to be a model of wound-repair response[6]. Carnevali *et al*. reported that cigarette smoke extract (CSE) inhibited fibroblast-mediated collagen gel contraction partly due to impaired fibronectin production from fibroblasts[7]. Epithelial cells, together with fibroblasts, are a primary component of airway wall structure. Epithelial cell plays a role as a barrier against exogenous toxic materials and has a protective function on cigarette-smoke induced airway injury[8, 9]. Epithelial cells may also interact with fibroblasts and extracellular matrices in tissue maintenance process. Furthermore, previous report showed human bronchial epithelial cells (HBEC) modulated collagen gel contraction by fibroblasts[10]

Glutathione (GSH) is a ubiquitous antioxidant in the lung epithelial cells and epithelial lining fluids (ELF). An increase of GSH level in the ELF of chronic smokers has been described[11], whereas this increase was not observed in the ELF under acute cigarette-smoke exposure[12]. There is little information about GSH levels in COPD patients. The extracellular GSH in the lungs protects alveolar macrophages, pulmonary epithelial cells, and pulmonary endothelial cells from oxidative stresses and helps maintain functional surfactant[13,14].

TGF-β1 is a potent mediator of fibrosis stimulating extracellular matrix (ECM) secretion and is involved in airway remodeling in COPD. Enhanced TGF-β1 expression in the bronchiolar and alveolar epithelium in COPD patients was reported[15]. Arsalane et al. have also shown that TGF-β1 can modulate GSH levels in alveolar epithelial cells in vitro by down-regulating expression of γ-glutamylcysteine synthetase (γ-GCS)[16]. In an animal experiment, lack of integrin $\alpha_V\beta_6$ resulted in MMP-12-dependent emphysema as a result of impaired TGF-β activation[17]. TGF-β is one of the most potent stimulators of ECM production and this fibrogenic growth factor is up-regulated in the lung parenchyma of patients with idiopathic pulmonary fibrosis. The lack of TGF-β activity would change ECM homeostasis in the lung to be resistant to fibrosis and this con-fibrotic condition could favor susceptibility to the development of COPD.

In the current study, we evaluated how alveolar epithelial cells and fibroblasts interact each other in the 3-D co-culture model under cigarette-smoke exposure by assessing gel contraction, levels of fibronectin, TGF-β1 and GSH.

Materials and Methods

Cell Culture

A normal human fetal lung fibroblast cell line (HFL-I) was obtained from RIKEN BioResource Center (Tsukuba-city, Ibaraki, Japan). A549, a human alveolar epithelial cell line, was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured in 75-cm^2 tissue culture flasks with Dulbecco's modified Eagle's medium (DMEM; SIGMA, St. Louis, MO, USA), supplemented with 10% fetal bovine serum (FBS), 50-U/ml penicillin and 50-μg/ml streptomycin. HFL-I and A549 were passed every 3 to 5 days. Confluent HFL-I and A549 were trypsinized (trypsin-EDTA; 0.05% trypsin, 0.53-mM EDTA-4Na; Gibco BRL, Grand Island, NY, USA) and the cells were used for 3-D culture.

Preparation of cigarette smoke extract (CSE)

Commercially available cigarettes (Seven Star®; Japan Tobacco Inc., Tokyo, Japan) were used, which are one of the most popular brands of filtered cigarettes in Japan. CSE was prepared by a modification of a previously published method[18]. Briefly, five filtered cigarettes, each containing 1.2 mg of nicotine and 14 mg of tar were smoked consecutively through an experimental apparatus with a constant airflow driven by a peristaltic pump. The smoke was bubbled through 10 ml of DMEM. Then the resulting suspension was filtered through 0.22-μm filter (Millipore, Bedford, MA, USA) to make standard CSE. The pH of the CSE prepared was kept between 7.4 and 7.5 in each experiment. CSE was freshly prepared just before each experiment.

Cell treatment

Three-dimensional collagen gel culture

Type I collagen from porcine skin (CELLGEN I-AC) was purchased from KOKEN Co. (Toshima-ku, Tokyo, Japan). Collagen gel was prepared by mixing distilled water, 4x concentrated serum-free DMEM, collagen solution and cell (HFL-I) suspension so that the final mixture was 0.6 mg/ml of collagen, 1x DMEM and 4.0 x 10^5 cells/ml (2.0 x 10^5 /gel). Immediately, 0.5-ml aliquots of this mixture were cast into each well of Falcon TM 24-well tissue culture plates (Becton Dickinson, Franklin Lakes, NJ, USA). After polymerizing

at 37°C for 30 minutes, 1-ml aliquots of A549 suspension in serum-free DMEM (2.0 X 10^5 cells/ml) were added to the wells for co-culture experiments, so that the cell density on top of the gel was 2.0 x 10^5 /gel. For HFL-I mono-culture controls, 1ml aliquots of serum-free DMEM were added into the wells instead of cell suspension. The gels were incubated for 2 hours at 37°C to let A549 attach to the top of the gels, and different concentration of CSE (0%, 2%, 5% and 10%) was added to the gels.

Gel contraction assay

For measuring gel contraction, the gels were released from the bottom of the wells after 24 hours from CSE exposure and kept floating in the wells. After 48 hours from CSE exposure, gel size was measured by taking photo-images of the culture plates using a flatbed scanner. The area of the gels was calculated using an image analyzing software Image J 1.30 (downloaded from http://rsb.info.nih.gov/). Gel contractility was expressed as the percentage of gel area compared with the initial gel area (%initial size). All cultures of each condition were performed in triplicate. Gels that were harvested from wells were stored at -20°C until the following assays. Media was also stored at -20°C until the following assays.

Total Glutathione (GSH) Quantification

Total intracellular glutathione (reduced glutathione and glutathione disulfide) in the gels was measured using Glutathione Quantification Kit (#T419, Dojindo Mo Tech Inc, Kumamoto-shi Kumamoto, Japan). After removing extra medium, gels were frozen and thawed twice in 0.9 ml of 10 mmol/L HCL solution to destroy the cell membrane. They were sonicated and immediately put on ice. Then, 0.1 ml of 5% 5-sulfosalicylic acid (SAA) was added to the gels to avoid oxidation of GSH and gels were centrifuged at 8000 g for 10 minutes at 4°C. The supernatants were transferred to other tubes and stored at -20°C until assayed. By incubating the supernatants with 5,5'-dithiobis 2-nitrobenzoic acid (DTNB), GSH was oxidized, accompanied with reduction of DTNB to 5-mercapto-2-nitrobenzoic acid, which was measured with a microplate reader by the absorbance at 405 nm. The concentration was calculated using a standard curve by GSH standard samples.

Measurement of TGF-β1 by ELISA

TGF-β1 release from the 3-D gels to culture media was quantified by ELISA with a commercially available kit (#DB100, R&D Systems, Minneapolis, MN, USA). The assay is isoform-specific and detects an epitope present in active form of TGF-β1. The samples harvested from 3-D collagen gels were assayed after acid activation and neutralization to measure total TGF-β1.

Measurement of fibronectin by ELISA

For quantification of fibronectin production from the 3-D collagen gels, the media surrounding the gels were harvested 48 hours after exposure to CSE. Fibronectin concentration was measured by ELISA using an assay kit (#MK115, TaKaRa Bio Inc, Ohtsu-shi, Shiga, Japan).

Effect of exogenous TGF-β1

To determine the effect of exogenous TGF-β on gel contraction, 0, 100 and 200 pM of recombinant human TGF-β1 (#240-B, R&D Systems, Minneapolis, MN, USA) and CSE (0%, 2%, 5% and 10%) was added in the media of the 3-D gels with A549 / HFL-I co-culture. After incubating for 48 hours, gel contraction was quantified and GSH concentration in the gel was measured by the same methods as previously described.

Statistical analysis

Statistical analysis was performed with Statcel software (Excel add-in software). All data are presented as mean $\pm$ standard error of the mean (SEM). Student's t-test was used for comparison of two groups. Multiple groups of the data were compared using the Kruskal-Wallis test. Bonferroni procedure was used to correct biases caused by multiple comparisons when statistical significances were identified in Kruskal-Wallis test. A value of $P<0.05$ was considered statistically significant.

Results

The effect of CSE on fibroblast-mediated collagen gel contraction

Collagen gel contraction was inhibited by CSE. 5% CSE completely inhibited contraction of gels containing only HFL-I. However, gel contraction was maintained in the gels containing both A549 and HFL-I up to 5% CSE. The most significant difference of contractility between two types of gels was observed at 5% CSE (Student's t-test , $^*P<0.05$, Figure 1). These data were derived from three separate experiments. Each experiment was done in triplicate and the three

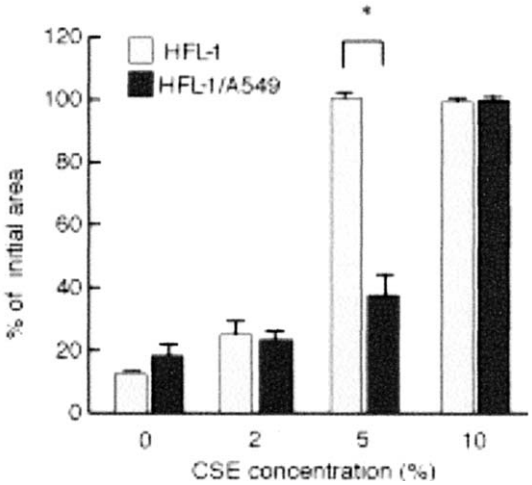

Fig. 1. Effect of CSE on fibroblast-mediated collagen gel contraction Exposure to 5% CSE completely inhibited contraction of gels containing HFL-I alone. However, gel contraction was maintained in the gels containing both A549 and HFL-I up to 5% CSE. The most significant difference of contractility between two types of the gels was observed at 5% CSE (**P*<0.05, Student's t-test). Data presented were from three separate experiments. Each experiment was performed in triplicate and the three results were averaged. Horizontal axis: concentration of CSE. Vertical axis: gel area expressed as percentage of initial size.

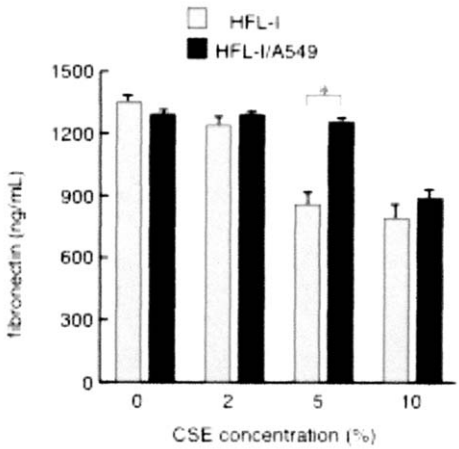

Fig. 2. Effect of CSE on fibronectin production in 3-D collagen gels. Fibronectin levels in the media surrounding the gels were maintained up to 5% CSE in the gels containing both A549 and HFL-I, while they were significantly reduced in the gels containing HFL-I alone. A significant difference was observed at 5% CSE (**P*<0.05, Student's t-test). Data presented were from five separate experiments. Each experiment was performed in triplicate and the three results were averaged. Exposure to 5% CSE reduced both fibronectin production and gel contraction by the gels with HFL-I alone, while the gels with A549 and HFL-I were affected only at 10% CSE exposure. Horizontal axis: concentration of CSE. Vertical axis: concentration of fibronectin.

results were averaged. The contractility was caused by HFL-I in this model, because A549 alone did not cause gel contraction without coexisting fibroblasts (data not shown), although previous report showed epithelial cells on the top of the gel contributed to gel contraction[19]. A549 itself is unlikely to have contributed directly to contractility, but probably affected the HFL-I-mediated contraction process.

Effect of CSE on fibronectin production in 3-D collagen gels

After incubation with CSE for 48hr, the supernatant media of 3-D gels was collected, and fibronectin was quantified by ELISA. Fibronectin production was reduced by CSE. Fibronectin levels in the media surrounding the gels were maintained at 5% CSE in the gels containing both A549 and HFL-I, while the levels were significantly reduced in the gels containing only HFL-I at 5% CSE exposure. Significant difference between two types of gels in the production of fibronectin was observed at 5% CSE (Student's t-test, **P*<0.05, Figure 2). Exposure to 5% CSE reduced both the fibronectin production and gel contraction by the gels with HFL-I alone, while the gels with both A549 and HFL-I were affected only at 10% CSE exposure. Even with 10% CSE exposure fibroblasts embedded in collagen gel released a certain amount of fibronectin (Figure 2).

Effect of CSE on GSH release

GSH levels in the gels containing only HFL-I were reduced by 5% CSE, and GSH was hardly detected at 10% CSE exposure. However, GSH levels in the gels containing both A549 and HFL-I were maintained even with 5% CSE exposure, while GSH level was reduced at 10% CSE (Figure 3). GSH production was synergistically increased when A549 and HFL-I coexisted, although GSH levels in A549 mono-culture were far lower than in the A549/HFL-I co-culture (data not shown). In addition, GSH levels were maintained up to 5% CSE exposure only in co-culture condition with both A549 and HFL-I. These data were from four separate experiments and each concentration of GSH was calculated from a sum total of three gels performed in triplicate.

Effect of CSE on TGF-β1 production

In the media surrounding the gels containing both A549 and HFL-I, TGF-β1 levels were more than twice as much as those of the gels containing HFL-I only at 0%, 2% and 5% of CSE (**P*<0.05, Figure 4). When only A549 was plated on top of the gels, TGF-β1 levels were

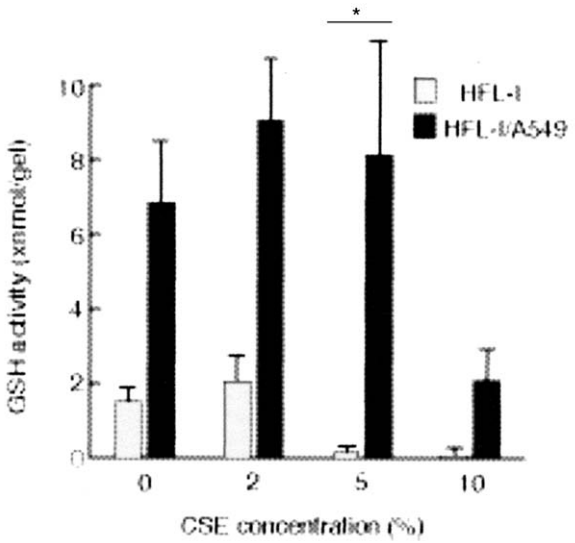

Fig. 3. Effect of CSE on GSH release.
In the 3-D gels containing HFL-I alone, GSH levels were quite low compared to the gels containing both A549 and HFL-I. GSH production was significantly increased when A549 and HFL-I coexisted at 5%CSE (*P<0.05, Bonferroni procedure). Data presented were from four separate experiments, and each experiment was done in triplicate. Horizontal axis: concentration of CSE. Vertical axis: GSH activity.

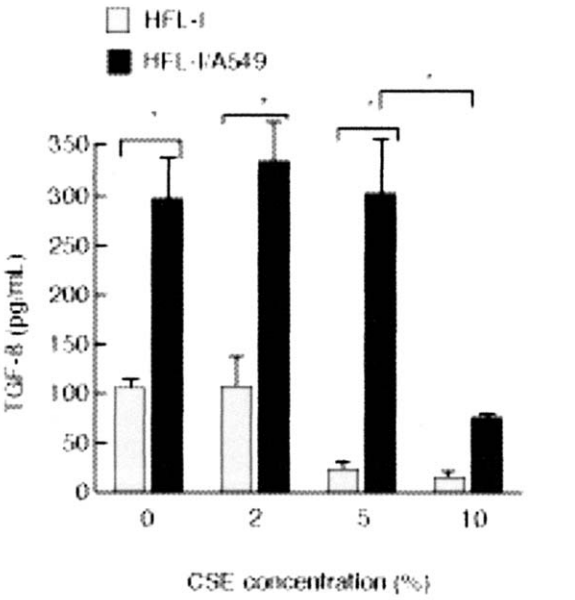

Fig. 4. Effect of CSE on TGF-β1 production
In the gels containing both A549 and HFL-I, TGF-β1 levels were more than twice as much as that of the gels containing HFL-I only (*P<0.05, Bonferroni procedure). Data presented were from three separate experiments, and each experiment was performed in triplicate. Horizontal axis: concentration of CSE. Vertical axis: concentration of TGF-β1.

similar to the 3-D gel with HFL-I alone at any concentration of CSE (control, 2%, 5%, and 10%) (data not shown). This suggests that there was an interaction between epithelial cells and mesenchymal cells resulting in synergistically augmented TGF-β1 production.

The effect of exogenous TGF-β1 on 3-D gels

Exogenous TGF-β1 augmented the contraction of the 3-D gels containing both A549 and HFL-I in a dose dependent manner at 2% and 5% of CSE (*P<0.05, Figure 5A). These data were derived from four separate experiments. Each experiment was done in triplicate and the three data were averaged.

Preliminary experiments revealed that gel contractility by 400pM of TGF-β1 was almost as equal as that by 200pM (data not shown). Therefore, 200pM of TGF-β1 was determined to be the optimum concentration for contracting the 3-D gels. In this experiment, TGF-β1 did not increase GSH production (Figure 5B). TGF-β1 reduced the inhibitory effect of CSE on gel contraction not by enhancing the neutralizing function through GSH but by enhancing the following contractile process mediated by fibroblasts.

Discussion

In the current study, we evaluated how alveolar epithelial cells and fibroblasts interact in the 3-D co-culture model under cigarette-smoke exposure by assessing gel contraction, levels of fibronectin, TGF-β1 and GSH.

Peripheral lung tissue is composed of fibroblasts, smooth muscle cells and capillary blood vessels embedded in ECM, covered with epithelial cell layer. We designed an in-vitro 3-D co-culture model to simulate lung tissue. Fibroblasts were embedded in type-I collagen gel lattice, and the surface of the gel was covered with a monolayer of epithelial cells. This culture system enables cross-talking between epithelial cells and mesenchymal cells through extracellular matrices independent of inflammatory cells, such as macrophages and neutrophils.

Fibroblast-mediated collagen gel contraction has been used as an *in-vitro* model of wound repair and mesenchymal tissue maintenance[20,21]. The gel contraction is a complex process, and CSE could affect several stages of contraction. The gel contraction was dependent on fibronectin and $\alpha_2\beta_1$-integrin which take part in binding fibroblasts with collagen, leading to the

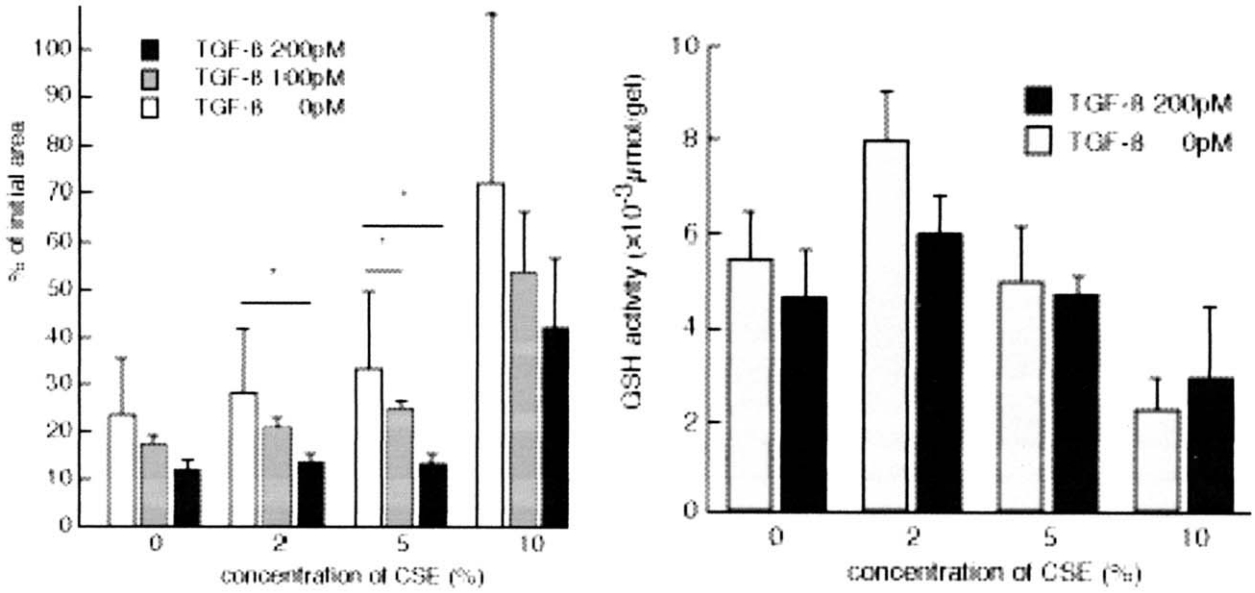

Fig. 5. Effect of exogenous TGF-β1 on collagen gel contraction and GSH release.
Co-cultured gel was employed in these experiments. (A) Exogenous TGF-β1 augmented the contraction of the 3-D gels containing both A549 and HFL-I in a dose dependent manner at 2% and 5%CSE (*P<0.05, Bonferroni procedure). Vertical axis: gel area expressed as percentage of initial size. (B) In this experiment, TGF-β1 did not increase GSH production. Horizontal axis: concentration of CSE. Vertical axis: GSH activity. (Data from four separate experiments and each experiment was performed in triplicate)

possible final process of the contraction[22]. In the current study, we first confirmed that CSE exposure reduced fibronectin production and inhibited collagen gel contraction by fibroblasts, suggesting that a decrease of fibronectin might have reduced gel contraction by inhibiting the binding of fibroblasts with collagen fibers. The co-existence of A549, an alveolar epithelial cell, reduced the inhibitory effect of CSE on the gel contraction at 5% CSE in our model. Although previous report showed that epithelial cells on the top of the 3-D gels contributed to gel contraction[19], A549 neither induced collagen gel contraction itself nor produced additional fibronectin in this experimental condition, suggesting that epithelial cells could protect fibroblasts from cigarette-smoke induced injury. The reason why A549 cells on top of the gels did not contribute to gel contraction in our preliminary experiments might be attributable to the different experimental conditions concerning the density of cells and serum-free cultures. The A549 might have contributed to the maintenance of gel contractility not by producing fibronectin but by producing other mediators that can either neutralize CSE or enhance fibroblast contractility. Fibronectin production was only partially reduced at 10% CSE, whereas gel contraction was completely inhibited at 10% CSE exposure. This observation indicates that fibroblast-mediated gel contraction was regulated not only by fibroblast-collagen binding through fibronectin, but by binding through $\alpha_2\beta_1$-integrin[23] or enhanced fibroblast contractility by α-smooth muscle actin (α-SMA). The last possibility is supported by the observation that fibroblasts are experimentally differentiated into myofibroblasts by cytokines such as TGF-β and α-SMA expression correlates with the strength of gel contraction[24].

Glutathione (GSH) is one of the most widely recognized antioxidants in the human lung, and is reported to have anti cigarette smoke activity. The current study suggested that A549 protected lung tissue from cigarette smoke-induced injury by releasing GSH. In addition, an interaction between A549 and HFL-I has led to a cumulative augmentation in producing GSH. In the co-culture model, GSH levels were augmented at 0 to 5% CSE, and were depleted at 10%, while GSH level in fibroblast alone was depleted at 5%. Arsalane et al. reported that acute cigarette smoke exposure depleted GSH in A549 cells in a monolayer culture[16]. In contrast, several in-vivo animal experiments showed that GSH levels returned to normal after an initial decrease induced by acute cigarette smoke exposure[25-28] suggesting the complex interactions between various cells in vivo. Fibroblasts might help epithelial cells pro-

duce GSH following cigarette smoke exposure in the co-culture system which is similar to the in-vivo process compared to the monolayer culture.

TGF-β is a potent functional regulator of fibroblasts by enhancing proliferation, inducing α-SMA expression and augmenting fibronectin production resulting in enhanced contractility. These activities must be important in mesenchymal tissue maintenance. TGF-β is known to be produced by both epithelial cells and fibroblasts. We found that TGF-β production was synergistically augmented, when epithelial cells and fibroblasts were co-cultured. 5% CSE inhibited TGF-β production from fibroblasts significantly, while CSE of the same concentration did not affect TGF-β production in A549 / HFL-I co-culture. Wang et al. reported that CSE inhibited TGF-β production from fibroblasts at low cell density leading to reduced gel contraction, while CSE did not affect TGF-β production at high cell density[29]. Addition of epithelial cells in the co-culture could cause a similar effect as was observed at high cell density in fibroblast monoculture suggesting that the two types of the cells work together to protect from cigarette smoke-induced injury. To determine the role of TGF-β1 in this culture system simulating alveolar wall, exogenous TGF-β1 was added to the co-culture model. Exogenous TGF-β1 restored the ability of fibroblasts to contract collagen gels impaired by cigarette smoke. Exogenous TGF-β1 restored the tissue repair process not by neutralizing cigarette smoke by augmentation of GSH release, but might affect on fibroblasts by increasing the contractility or enhancing the fibroblast-collagen binding through fibronectin or other adhesion systems leading to augmented cell proliferation and cell differentiation. Therefore, up-regulation of TGF-β1 activity inhibits progressive destruction of the lung tissue in COPD and may be a candidate of therapeutic approach for COPD.

In summary, we designed an in vitro 3-D co-culture model, which contained alveolar epithelial cells and fibroblasts. The presence of epithelial cells prevented the inhibition of fibroblast-mediated gel contraction by CSE and restored production of GSH, TGF-β1 and fibronectin. These observations suggested that epithelial cells protected fibroblasts from cigarette-smoke induced injury. Epithelial cells released GSH even in the presence of CSE in the co-culture condition, in contrast to the mono-culture experiments previously reported. Gel contraction, production of GSH, TGF-β1 and fibronectin are seemed to be a series of sequential changes in this model. TGF-β1 is likely to be most important for the consequences in this 3-D culture model. This 3-D model co-cultured with A549 and HFL-I may be a useful model for studying the pathology of COPD independent from inflammatory process.

Acknowledgements

We thank Dr. Vernon L. Moore for his critical review of the manuscript. Also we thank Dr. Wataru Ohashi and Professor Hiroshi Tanaka, Department of Bioinformatics, Tokyo Medical and Dental University, for the advise of statistical analysis.

References

1. Hautamaki RD, Kobayashi DK, Senior RM, et al. Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice. Science 1997;277(5334): 2002-4.
2. Aoshiba K, Yokohori N, Nagai A. Alveolar wall apoptosis causes lung destruction and emphysematous changes. Am J Respir Cell Mol Biol 2003;28(5):555-62.
3. Kasahara Y, Tuder RM, Cool CD, et al. Endothelial cell death and decreased expression of vascular endothelial growth factor and vascular endothelial growth factor receptor 2 in emphysema. Am J Respir Crit Care Med 2001;163 (3 Pt 1): 737-44.
4. Kasahara Y, Tuder RM, Taraseviciene-Stewart L, et al. Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. J Clin Invest 2000;106(11):1311-9.
5. Rennard SI. Inflammation and repair processes in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1999;160(5 Pt 2):S12-6.
6. Grinnell F. Fibroblasts, myofibroblasts, and wound contraction. J Cell Biol 1994;124(4):401-4.
7. Carnevali S, Nakamura Y, Mio T, et al. Cigarette smoke extract inhibits fibroblast-mediated collagen gel contraction. Am J Physiol 1998;274(4 Pt 1):L591-8.
8. Brown LA. Glutathione protects signal transduction in type II cells under oxidant stress. Am J Physiol 1994;266(2 Pt 1): L172-7.
9. Brown LA, Bai C, Jones DP. Glutathione protection in alveolar type II cells from fetal and neonatal rabbits. Am J Physiol 1992;262(3 Pt 1):L305-12.
10. Mio T, Liu XD, Adachi Y, et al. Human bronchial epithelial cells modulate collagen gel contraction by fibroblasts. Am J Physiol 1998;274(1 Pt 1):L119-26.
11. Cantin AM, North SL, Hubbard RC, et al. Normal alveolar epithelial lining fluid contains high levels of glutathione. J Appl Physiol 1987;63(1):152-7.
12. Morrison D, Rahman I, Lannan S, et al. Epithelial permeability, inflammation, and oxidant stress in the air spaces of smokers. Am J Respir Crit Care Med 1999;159(2):473-9.
13. Cross CE, van der Vliet A, O'Neill CA, et al. Oxidants, antioxidants, and respiratory tract lining fluids. Environ Health Perspect 1994;102 Suppl 10:185-91.
14. Hagen TM, Brown LA, Jones DP. Protection against paraquat-induced injury by exogenous GSH in pulmonary alveolar type II cells. Biochem Pharmacol 1986;35(24):4537-42.

15. de Boer WI, van Schadewijk A, Sont JK, et al. Transforming growth factor beta1 and recruitment of macrophages and mast cells in airways in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1998;158(6):1951-7.
16. Arsalane K, Dubois CM, Muanza T, et al. Transforming growth factor-beta1 is a potent inhibitor of glutathione synthesis in the lung epithelial cell line A549: transcriptional effect on the GSH rate-limiting enzyme gamma-glutamylcysteine synthetase. Am J Respir Cell Mol Biol 1997;17(5):599-607.
17. Morris DG, Huang X, Kaminski N, et al. Loss of integrin alpha(v)beta6-mediated TGF-beta activation causes Mmp12-dependent emphysema. Nature 2003;422(6928): 169-73.
18. Hoshino Y, Mio T, Nagai S, et al. Cytotoxic effects of cigarette smoke extract on an alveolar type II cell-derived cell line. Am J Physiol Lung Cell Mol Physiol 2001;281(2):L509-16.
19. Umino T, Wang H, Zhu Y, et al. Modification of type I collagenous gels by alveolar epithelial cells. Am J Respir Cell Mol Biol 2000;22(6):702-7.
20. Bell E, Ivarsson B, Merrill C. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. Proc Natl Acad Sci U S A 1979;76(3):1274-8.
21. Mio T, Adachi Y, Romberger DJ, et al. Regulation of fibroblast proliferation in three-dimensional collagen gel matrix. In Vitro Cell Dev Biol Anim 1996;32(7):427-33.
22. Klein CE, Dressel D, Steinmayer T, et al. Integrin alpha 2 beta 1 is upregulated in fibroblasts and highly aggressive melanoma cells in three-dimensional collagen lattices and mediates the reorganization of collagen I fibrils. J Cell Biol 1991;115(5):1427-36.
23. Riikonen T, Koivisto L, Vihinen P, et al. Transforming growth factor-beta regulates collagen gel contraction by increasing alpha 2 beta 1 integrin expression in osteogenic cells. J Biol Chem 1995;270(1):376-82.
24. Hinz B, Celetta G, Tomasek JJ, et al. Alpha-smooth muscle actin expression upregulates fibroblast contractile activity. Mol Biol Cell 2001;12(9):2730-41.
25. Bilimoria MH, Ecobichon DJ. Protective antioxidant mechanisms in rat and guinea pig tissues challenged by acute exposure to cigarette smoke. Toxicology 1992;72(2):131-44.
26. Cotgreave IA, Johansson U, Moldeus P, et al. The effect of acute cigarette smoke inhalation on pulmonary and systemic cysteine and glutathione redox states in the rat. Toxicology 1987;45(2):203-12.
27. Ishizaki T, Kishi Y, Sasaki F, et al. Effect of probucol, an oral hypocholesterolaemic agent, on acute tobacco smoke inhalation in rats. Clin Sci (Lond) 1996;90(6):517-23.
28. Li XY, Rahman I, Donaldson K, et al. Mechanisms of cigarette smoke induced increased airspace permeability. Thorax 1996;51(5):465-71.
29. Wang H, Liu X, Umino T, et al. Effect of cigarette smoke on fibroblast-mediated gel contraction is dependent on cell density. Am J Physiol Lung Cell Mol Physiol 2003;284(1):L205-13.