Efficacies of rosiglitazone and retinoin on bleomycin-induced pulmonary fibrosis in rats

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Received December 11, 2015; Accepted January 26, 2017

DOI: 10.3892/etm.2017.4555

Abstract. The present study investigated the intervention efficacies of rosiglitazone (ROS) and retinoin (RET) on bleomycin-induced pulmonary fibrosis in rats. A total of 48 rats were randomly divided into the control group (group C), the model group (group M), the dexamethasone group (group D), the ROS group (group R), the RET group (group W) and the ROS + RET group (group L). Group M and the treatment groups were intratracheally injected with 5 mg/kg bleomycin, while group C was injected with saline. The lungs of rats in each group were inspected using high resolution computed tomography (HRCT), lung tissue hematoxylin and eosin staining and Masson staining; furthermore, lung L-hydroxyproline (Hyp) content and the concentration of transforming growth factor β1 (TGF-β1) serum of each group were also determined. The fibrosis score, Hyp content and TGF-β1 concentration of each treatment group were significantly lower when compared with group M ($P<0.01$), while the imaging results were improved when compared with group M, with lower alveolitis and fibrosis scores. Group L, R and W exhibited significantly lower fibrosis scores, Hyp content and TGF-β1 concentrations when compared with group D ($P<0.05$). Imaging results for group L, R and W indicated that while the imaging results were superior to group D, group L was lower than groups R and W ($P<0.05$). No significant difference in the fibrosis score, Hyp content and TGF-β1 concentration was exhibited between groups R and W ($P>0.05$). Findings from the present study conclude that ROS and RET are able to suppress bleomycin-induced pulmonary fibrosis with improved efficacies when compared with dexamethasone; furthermore, the combination of these two pharmacological agents may exert synergistic effects.

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Key words: bleomycin, pulmonary fibrosis, rosiglitazone, retinoin

Introduction

Idiopathic pulmonary fibrosis (IPF) is an unexplained chronic fibrotic interstitial pneumonia, as well as the main type of idiopathic interstitial pneumonia (IIP). In recent years, its prevalence and annual incidence rate has significantly increased (1). The lung histology and/or chest high-resolution computed tomography (HRCT) of IPF has been characterized by usual interstitial pneumonia (UIP) in previous studies (2,3). IPF exhibits unknown etiology and poor prognosis, with a median survival period of only 2 to 3 years following diagnosis and there is a current lack of satisfactory treatment available (4,5). The combination of prednisone, azathioprine and N-acetylcysteine has been demonstrated to have little effect on hindering disease progression in patients with IPF; however, multiple side effects may occur (6). Furthermore, N-acetylcysteine monotherapy is not able to suppress the acute exacerbation and reduce the mortality of patients with IPF (7). Pirfenidone possesses anti-inflammatory, anti-fibrotic and anti-oxidant properties and has been indicated to impede the declining rate of lung functions, but it has little benefit on the mortality of the disease (8). Therefore, considering more effective agents for the treatment of IPF should be a predominant focus. As a synthetic ligand, rosiglitazone (ROS) is the representative agonist of peroxisome proliferator-activated receptor γ (PPARγ). PPARγ and its ligand participate in lipid and sugar metabolism, immune and inflammatory responses and exert anti-fibrosis effects in multiple organs (9-11). Retinoin (RET) is the product of oxidative metabolism of vitamin A *in vivo* and exhibits regulatory roles for a variety of immune and inflammatory responses. RET is able to promote the repair of alveolar epithelial cells (12,13). To date, few reports have indicated the anti-pulmonary fibrosis roles of ROS (5). The present study observed the *in vivo* intervention effects and efficacies of ROS and RET on bleomycin-induced pulmonary fibrosis in rats.

Materials and methods

Animal grouping and model preparation. A total of 48 healthy male Wistar rats, 2 months old, weighing 220±23 g, were purchased from Qingdao Institute for Food and Drug Control (Qingdao, China), had free access to food and water and were maintained in an environment at a temperature of 22˚C.

Rats were randomly divided into control (group C), model (group M), dexamethasone (group D), ROS (group R), RET (group W) and ROS + RET (group L) groups. Group C received 0.3-0.4 ml of saline which was slowly intratracheally instilled, while 0.3-0.4 ml bleomycin A5 saline solution (5 mg/kg; CLEA Japan, Inc., Tokyo, Japan) was slowly intratracheally instilled to prepare the model of bleomycin-induced pulmonary fibrosis. All groups received consecutive 31-day oral administration and intraperitoneal injection 24 h following modeling. Group C was intraperitoneally injected with an 5 mg/kg saline + oral administration of saline; group M was intraperitoneally injected the equal amount of saline + oral administration of saline; group D was intraperitoneally injected with 5 mg/kg dexamethasone (Jiangsu Lianshui Pharmaceutical Co., Ltd., Lianshui, China) + an equal amount of oral saline; group R was orally administered 4 mg/kg ROS + intraperitoneally injected saline (equal amount); group W was orally administered 20 mg/kg RET (Jiangsu Lianshui Pharmaceutical Co., Ltd.) + intraperitoneally injected saline (equal amount); and group L was orally administered 4 mg/kg ROS (Chongqing Fuling Pharm., Taiji Group, China) + 20 mg/kg RET + intraperitoneally injected saline (equal amount). The present study was performed in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Qingdao University (Qingdao, China).

Specimen collection. Rats from each group were inspected using HRCT on days 7, 21 and 31, respectively, followed by bone algorithm and standard algorithms for the reconstruction. Two rats from each group were randomly selected and sacrificed on day 7, while the rest rats were all sacrificed on day 31.

Hematoxylin and eosin (H&E) staining and Masson staining. Lung tissue sections from sacrificed rats were fixed with 4% formalin for 24 h, paraffin embedded, sectioned (5-μm thick) and exposed to H&E staining and Masson staining and the morphological changes of lung tissues were observed using a light microscope (magnification, x200). The degrees of alveolitis and pulmonary fibrosis were determined as outlined by Szapiel *et al* (14).

Alkaline hydrolysis method. The alkaline hydrolysis method was used to detect the hydroxyproline (Hyp) content in lung tissues. A spectrophotometer (Shanghai Analytical Instrument Co., Shanghai, China) was used to measure the absorbance of each sample tube. Hyp content was subsequently calculated according to the following formula: Hyp content=(absorbance of sample tube-absorbance of blank tube)/(absorbance of standard tube-absorbance of blank tube) x content of standard tube (5 μg/ml) x total volume of hydrolyzate (5 ml)/tissue wet weight (mg).

Detection of serum transforming growth factor-β1 (TGF-β1). ELISA assay (48 t; Wuhan Boster biotechnology Co., Wuhan, China) was performed to detect serum TGF-β1 concentration, according to the manufacturer's instructions.

Statistical analysis. Experimental data were processed using SPSS 17.0 statistical software (SPSS, Inc., Chicago, IL, USA). Data were expressed as the mean ± standard deviation. Grade data were transformed into the measurement data: 0 point for grade 0, 1 point for grade 1, 2 points for grade 2, 3 points for grade 3, and so on. Intergroup comparison was performed using Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Appearance. Rats in group C were generally in good condition; however, rats in group M were listless and tired, and suffered from mild dyspnea and cyanosis of their peripheral limbs and lips, and the rats' activities decreased: The overall mental status, food intake, body weight, colors of extremities and lips and greater levels of motility were observed in R, W and L groups when compared with group M; while rats in group D were emaciated and their abilities of disease-resistance were weak.

Pathological observation. H&E staining revealed that rats in group C exhibited normal lung tissue structures at each observation time point. However, the alveolar space of the rats in group M exhibited marked inflammatory exudation and inflammatory cell infiltration on day 7 following modeling. Furthermore, the alveolar septum was widened and rare fibroblasts were observed. The inflammatory response in each intervention group was mild (Fig. 1). The alveolitis scores and performance of each group are indicated in Table I. On day 31, no evidence of inflammatory cell infiltration was apparent in group M; however, the alveolar structures were disordered, the alveolar wall and alveolar septum were thickened and an increased number of mature fibroblasts were accumulated inside. Furthermore, the red-stained fibrous tissues were proliferated and the number of matrix components was significantly increased. All intervention groups (R, W and L groups) exhibited essentially identical characteristics of mild inflammation and fibrosis as group M at each time point. Masson staining revealed that the lung tissue structures in group C were clear and complete at each time point; however, at different time points (on day and 31) an increased amount of blue collagen was deposited in the alveolar septum, bronchi and perivascular areas in group M. Over time, the collagen content increased. However, blue collagen deposition in the alveolar septum, bronchi and perivascular areas in group R, W and L groups were mild. Fibrosis scores of all intervention groups were significantly decreased when compared with group M ($P<0.01$). Fibrosis scores of groups L, R and W were significantly decreased when compared with group D ($P<0.05$). No significant difference in fibrosis scores was observed between groups R and W; however, the score of group L was significantly decreased when compared with groups R and W ($P<0.05$; Fig. 2). The pulmonary fibrosis score and performance of each group on day 31 is indicated in Table II.

Hyp content and serum TGF-β1 concentration. The present study identified that the Hyp content and serum TGF-β1 concentration in the lung tissues of group M were significantly increased when compared with group C ($P<0.01$). Hyp content

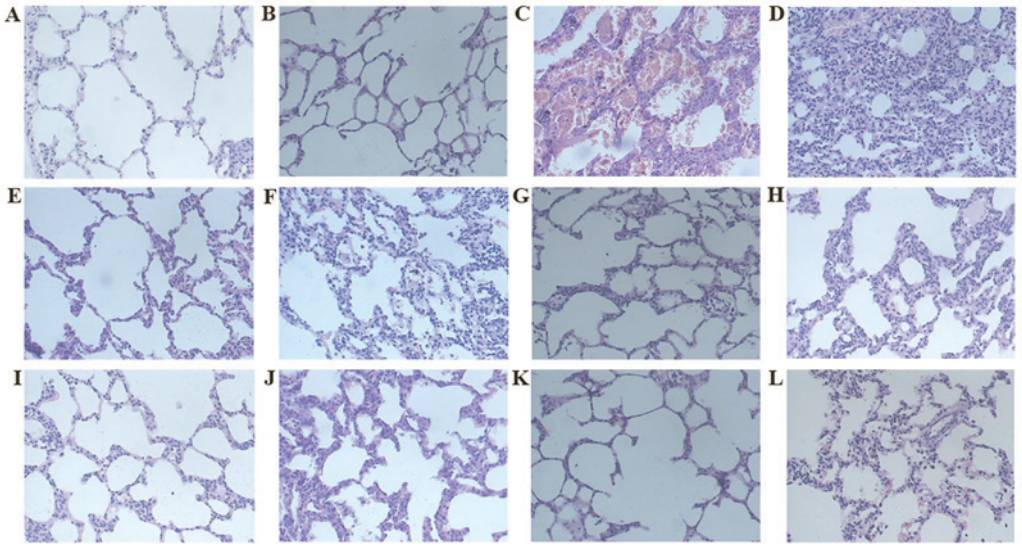

Figure 1. H&E staining (magnification, x200). Group C on days (A) 7 and (B) 31, respectively. Group M on days (C) 7 and (D) 31, respectively. Group D on days (E) 7 and (F) 31, respectively. Group R on days (G) 7 and (H) 31, respectively. Group W on days (I) 7 and (J) 31, respectively. Group L on days (K) 7 and (L) 31, respectively. H&E staining revealed that rats in Group C exhibited normal lung tissue structures at each observation time point; while the alveolar space of the rats in group M exhibited markedly increased inflammatory exudation and inflammatory cell infiltration on day 7 following modeling. The alveolar septum was widened, while rare fibroblasts were seen; the inflammation situation in groups L, R and W was mild. On day 31, no inflammatory cell infiltration in group M was apparent and the alveolar structures were disordered; the alveolar wall and alveolar septum were thickened and a large number of mature fibroblasts had accumulated inside. The red-stained fibrous tissues proliferated, and the number of matrix components had markedly increased. All intervention groups exhibited essentially identical characteristics of mild inflammation and fibrosis as group M at each time point. H&E, hematoxylin and eosin; ROS, rosiglitazone; RET, retinoin; group C, the control group; group M, the model group; group D, the dexamethasone group; group R, the ROS group; group W, the RET group; group L, the ROS + RET group.

Table I. Alveolitis score of each group on day 7 (mean ± standard deviation).

Group	Alveolitis score
C	0±0.10
M	2.5±0.52[a]
D	1.5±0.22[a,b]
R	1.0±0.16[a-c]
W	1.0±0.14[a-c]
L	0.5±0.13[a,b,d]

[a]P<0.05 vs. group C, t=6.6768, 8.7781, 7.4953, 8.2199, 4.3113; [b]P<0.05 vs. group M, t=3.0676, 4.7754, 4.8245, 6.4628; [c]P<0.05 vs. group D, t=2.5994, 2.7116, 4.1536; [d]P<0.05 vs. groups R and W, t=3.4300, 3.7012. ROS, rosiglitazone; RET, retinoin; group C, the control group; group M, the model group; group D, the dexamethasone group; group R, the ROS group; group W, the RET group; group L, the ROS + RET group.

Table II. Fibrosis score of each group on day 31 (mean ± standard deviation).

Group	Fibrosis score
C	0.167±0.230
M	2.833±1.213[a]
D	2.500±0.921[a,b]
R	1.400±0.412[a-c]
W	1.200±0.458[a-c]
L	0.833±0.331[a-d]

[a]P<0.01 vs. group C, t=5.2894, 6.0992, 6.2890, 4.8718, 4.0474; [b]P<0.01 vs. group M, t=2.2766, 2.5049, 2.8260, 3.8963; [c]P<0.05 vs. group D, t=2.4163, 2.7873, 4.1536; [d]P<0.05 vs. groups R and W, t=2.5362, 2.2410. ROS, rosiglitazone; RET, retinoin; group C, the control group; group M, the model group; group D, the dexamethasone group; group R, the ROS group; group W, the RET group; group L, the ROS + RET group.

and serum TGF-β1 concentration in each intervention group were significantly decreased when compared with group M (P<0.01). Compared with group D, the Hyp content and serum TGF-β1 concentration in the lung tissues of group L, R and W were significantly decreased (P<0.05); however, Hyp content and serum TGF-β1 concentration of groups R and W exhibited no significant difference (P>0.05). Furthermore, the Hyp content and serum TGF-β1L concentration in the lung tissues of group L were significantly increased when compared with group R and W (P<0.05; Table III).

HRCT signs. Imaging results of rats in group C exhibited a clear outline of the whole lung with uniformly distributed lung markings and a regular interface; the alveolar septal thickness was normal and the lung septal and lobular structures were normal. Group M exhibited the typical changes of alveolitis-pulmonary fibrosis over a period of time, including ground glass opacity and consolidation on day 7, which may prompt the phase of alveolitis; additionally, honeycombing was observed on day 31. Improved radiological signs of varying degrees were observed in the intervention groups

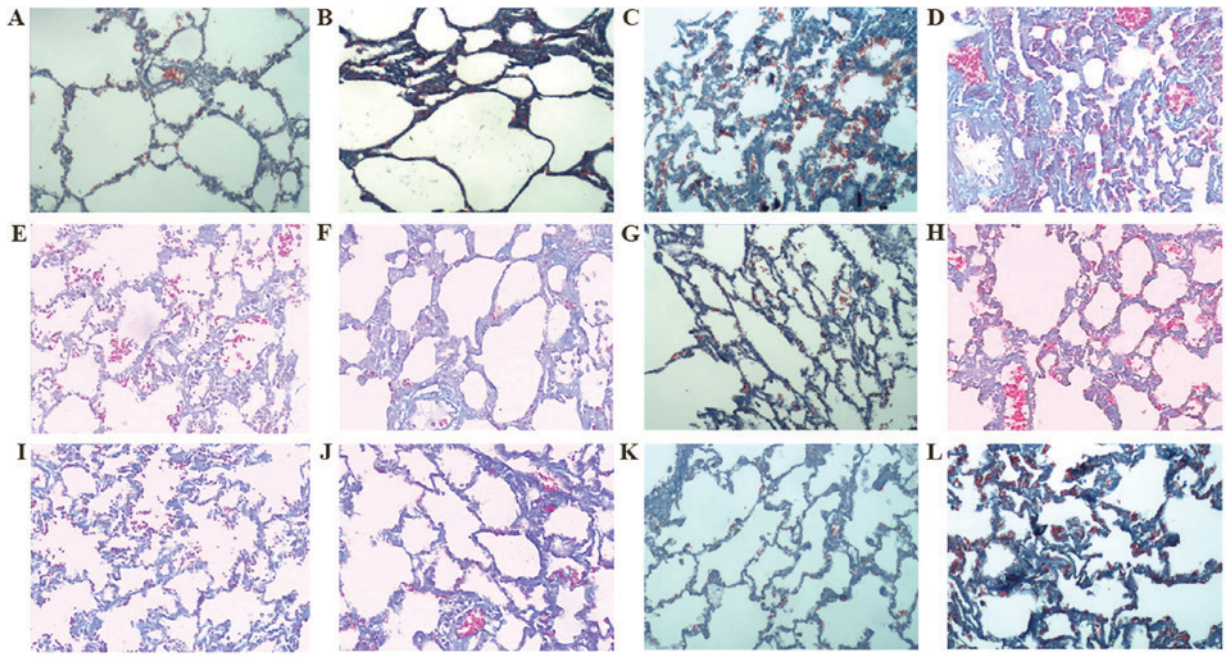

Figure 2. Masson staining (magnification, x200). Group C on days (A) 7 and (B) 31, respectively. Group M on days (C) 7 and (D) 31, respectively. Group D on days (E) 7 and (F) 31, respectively. Group R on days (G) 7 and (H) 31, respectively. Group W on days (I) 7 and (J) 31, respectively. Group L on days (K) 7 and (L) 31, respectively. Masson staining revealed that the lung tissue structures in group C were clear and complete at each time point, while at different time points, an abundance of blue collagens were deposited in the alveolar septum, bronchi and perivascular areas in group M suggesting that over time, the quantity of collagens increased. As outlined in Table II, the fibrosis scores of all intervention groups were significantly decreased when compared with group M ($P<0.01$). Group L, R and W exhibited decreased fibrosis scores when compared with group D ($P<0.05$). No significant difference in fibrosis score was observed between groups R and W, while the score of group L was significantly decreased when compared with groups R and W ($P<0.05$). ROS, rosiglitazone; RET, retinoin; group C, the control group; group M, the model group; group D, the dexamethasone group; group R, the ROS group; group W, the RET group; group L, the ROS + RET group.

Table III. Hyp content and serum TGF-β1 of lung tissues from different group (mean ± standard deviation).

Group	N (rats)	Hyp (μg/g)	TGF-β1 (pg/ml)
C	6	1853.6±297	9.78±4.96
M	6	4863.2±546[a]	37.82±9.89[a]
D	4	3962.5±298[a,b]	17.2l±3.10[a,b]
R	5	3122.9±316[a-c]	15.32±2.83[a-c]
W	5	3232.1±293[a-c]	14.36±3.22[a-c]
L	6	2530.9±316[a-d]	12.71±4.38[a-d]

[a]$P<0.01$ vs. group C, t1=(11.8606, 10.9854, 6.8594, 7.7110, 3.8256) t2=(6.2078, 2.6421, 2.3763, 2.2971, 2.0198); [b]$P<0.01$ vs. group M, t1=(2.9775, 6.2716, 5.9672, 9.0559) t2=(4.8709, 5.3576, 5.5250, 5.6864); [c]$P<0.05$ vs. group D, t1=(4.0582, 3.6890, 7.1688) t2=(2.1029, 2.5619, 2.0542); [d]$P<0.05$ vs. groups R and W, t1=(3.2449, 3.9857) t2=(2.2260, 2.5521). ROS, rosiglitazone; RET, retinoin; group C, the control group; group M, the model group; group D, the dexamethasone group; group R, the ROS group; group W, the RET group; group L, the ROS+RET group; Hyp, L-hydroxyproline; TGF-β1, transforming growth factor β1.

when compared with group M at the same time point. The degree of fibrosis exhibited in the images of groups L, W and R was decreased when compared with group D, indicating that RET and ROS may improve bleomycin-induced pulmonary fibrosis to some extent (Fig. 3). Radiographic scores are outlined in Table IV and the HRCT images are indicated in Fig. 3.

Discussion

The method of intratracheally injecting bleomycin to construct a pulmonary fibrosis model in rats is relatively simple and its pathophysiological changes and processes are the most similar to those in human pulmonary fibrosis (15). Following preparation of the bleomycin model, the pathology of the lung tissues exhibited severe alveolitis on day 7 and, according to the Szapiel classification (14), the majority of the rats exhibited grade 3 alveolitis, while the majority of inflammatory cells were no longer observed on day 31. Furthermore, collagen deposition was apparent, indicating that the states of fibrosis were predominantly distributed at grades 3-4. These pathological changes identified in the present study are consistent with those stated in the literature (16), indicating that the animal model was successfully established. In order to objectively evaluate the interventional effects of each pharmacological agent, light microscopic histological observation and imaging HRCT observation were conducted. The contents of lung tissue Hyp and serum TGF-β1 were also detected. Histological and imaging observations were intuitive and allowed for lesions to be located by the unique amino acid of collagen fibers. Detected Hyp content in lung tissues was converted to calculate the content of collagen and was recognized as the gold standard for pulmonary fibrosis. TGF-β1 has been indicated to promote and stimulate

Table IV. Changes and counts of radiological signs in each group.

Group	Day	N	LC	VBB	II	PT	GGO	N	H	Sum
C	7	2	0	0	0	0	0	0	0	0
	21	2	0	0	0	0	0	0	0	0
	31	2	0	0	0	0	0	0	0	0
M	7	2	2	0	0	0	2	1	0	3
	21	2	0	1	1	1	0	2	2	7
	31	6	1	3	4	3	2	3	4	20
D	7	2	1	0	0	0	2	0	0	3
	21	2	1	1	0	1	1	2	0	6
	31	4	1	2	3	2	2	2	2	16
R	7	2	1	0	0	0	1	0	0	2
	21	2	0	1	1	1	0	0	0	3
	31	5	2	3	1	1	1	3	0	11
W	7	2	2	1	0	0	0	0	0	1
	21	2	0	1	1	0	0	1	0	3
	31	5	1	2	1	1	1	1	0	7
L	7	2	2	0	0	0	0	1	0	1
	21	2	0	1	0	0	0	1	0	1
	31	6	1	2	1	0	0	2	0	6

Grade data were transformed into the measurement data: 0 points for grade 0, 1 point for grade 1, 2 points for grade 2, 3 points for grade 3, 4 points for grade 4, 5 points for grade 5, 6 points for grade 6. LC, lung consolidation; VBB, vascular bronchial bundle; II, irregular interface; PT, pleural thickening; GGO, ground glass opacities; N, nodular; H, honeycombing; ROS, rosiglitazone; RET, retinoin; Group C, the control group; group M, the model group; group D, the dexamethasone group; group R, the ROS group; group W, the RET group; group L, the ROS + RET group.

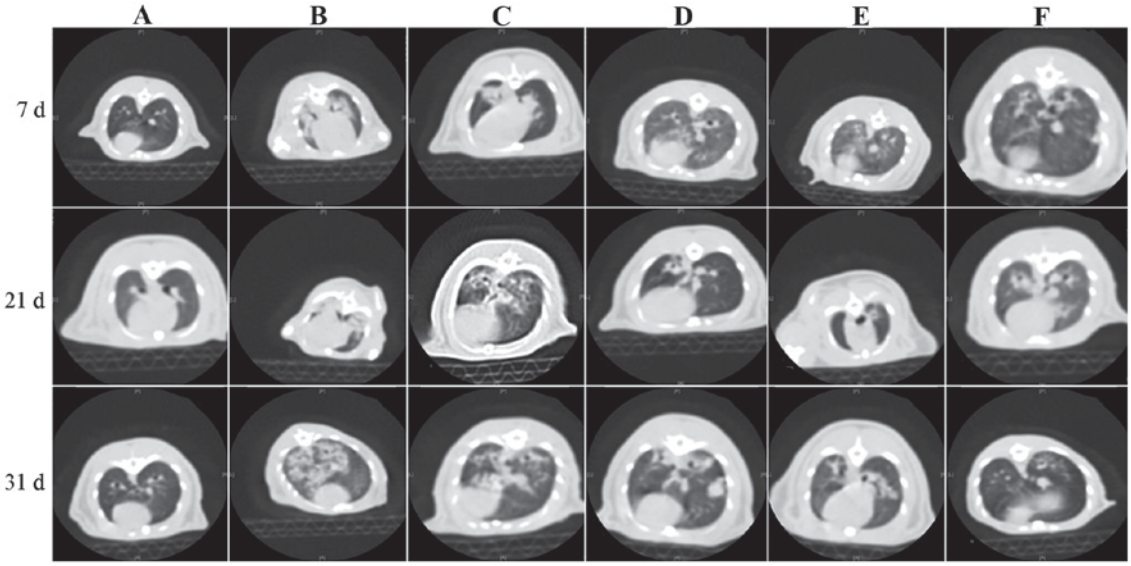

Figure 3. Histology and/or chest high-resolution computed tomography on day 7, 21 and 31. Day 7: (A) Lungs of normal rat; (B) bronchial vascular bundle of group M was thickened, and exhibited consolidated shadows; (C) group D exhibited decreased pulmonary transmittance and consolidated shadows; (D) group R exhibited ground glass-like shadows in two lungs; and (E) group W exhibited ground glass-like shadows in two lungs; (F) group L exhibited reduced pulmonary transmittance and small patchy shadows under the pleura. Day 21: (A) Lungs of normal rat; (B) group M exhibited large area of consolidated shadows in bilateral lungs, which was bigger than week 1; (C) group D exhibited thickened bronchial vascular bundle, with shallow pale ground-glass shadows inside the lungs; (D) group R exhibited thickened pulmonary bronchial vascular shadows and blurred edges; (E) group W exhibited pulmonary consolidated shadows plus cavities; and (F) group L exhibited thickened bronchial vascular bundle and blurred edges. Day 31: (A) Lungs of normal rat; (B) group M exhibited pulmonary increased density shadows, with small cavities similar to honeycomb-like changes inside; (C) group D exhibited rough and messy bronchial vascular bundles and multiple nodular shadows inside the lungs; (D) group R exhibited thickened bronchial vascular bundle and nodular shadows; (E) group W exhibited thickened bronchial vascular bundle and patchy shadows; and (F) group L exhibited pale patchy shadows. ROS, rosiglitazone; RET, retinoin; group C, the control group (group C); group M, the model group; group D, the dexamethasone group; group R, the ROS group; group W, the RET group; group L, the ROS + RET group.

the release of a variety of pro-fibrotic cytokines from macrophages, thus promoting the collagen deposition inside lung tissues (17,18). The combination of various methods may ensure the objectivity and accuracy of experimental results.

PPARγ is a member of the PPAR family, which is part of the nuclear receptor superfamily (9). Previous studies have indicated that PPARγ and its ligand may participate in the metabolism of lipids and sugar; furthermore, they have been implicated in growth, reproduction, immunity and inflammation-related reactions and exhibit anti-fibrotic effects on multiple organs, such as the kidney, liver and heart (9-11). As a synthetic ligand, ROS is a typical PPARγ agonist and is a well-established treatment for type 2 diabetes (9). *In vitro* experiments have indicated that PPARγ agonists may inhibit the chemotaxis of inflammatory cells, such as macrophages. PPARγ agonists have also been suggested to inhibit the production of inflammatory cytokines, reduce inflammation and block the occurrence of inflammatory responses (10). A previous study revealed that PPARγ was expressed in bronchial and alveolar epithelial cells, vascular endothelial cells, fibroblasts and alveolar macrophages (19), suggesting that this receptor is closely associated with the development of lung diseases. Furthermore, PPARγ has been indicated to elicit anti-inflammatory and anti-fibrosis effects in lung diseases. Therefore, PPARγ agonists may not only regulate inflammatory responses and anti-fibrotic effects but may also inhibit the expression of TGF-β1, thereby inhibiting the proliferation of fibroblasts and their conversion to myofibroblasts and the synthesis of collagens, thus reducing the occurrence of fibrosis (10,12). Burgess *et al* (20) and Genovese *et al* (21) have demonstrated this phenomenon through *in vitro* experiments. Additionally, Milam *et al* (22) demonstrated that the administration of PPARγ agonists following the formation of pulmonary fibrosis in rats may provoke anti-pulmonary fibrosis effects.

The morphologies of groups R and D on day 7 showed that the states of alveolitis in lung tissues were significantly decreased when compared with group M, suggesting that ROS and dexamethasone are able to decrease the effects of inflammation early on. In addition, the fibrosis score of group R on day 31 was decreased when compared with groups M and D, and HRCT revealed the rats exhibited mild fibrosis, which was an improvement compared with the other two groups, suggesting that ROS had an inhibitory effect on advanced fibrosis. The Hyp content and serum TGF-β1 concentration in the lung tissues of group R was significantly decreased in group R compared with groups M and D, suggesting that ROS may activate the PPARγ pathway, thus inhibiting the transcription and expression of TGF-β1 and subsequently reducing the transformation of fibroblasts to myofibroblasts and reducing collagen deposition.

RET is the most active derivative of vitamin A, which acts as a gene expression regulatory factor that is able to bind with specific receptors, thus regulating the expression of related target genes and controlling matrix metabolism, cell growth and differentiation (23). RET receptors are present in almost all cells (23). Chinese and Western researchers have identified that RET has positive roles in fighting against fibrosis in bone marrow, liver, kidney and other organ systems, in which RET mediates interstitial fibrosis by predominantly reducing the expression of TGF-β1 inside the interstitial tissues, thus reducing the accumulation of extracellular collagen (13,24,25). RET may inhibit the collagen synthesis process in human lung fibroblasts, both in a steady state and following TGF-β1 stimulation (26). A prior report revealed that, following the activation of RET receptors, alveolar damage in rats was reduced and normal lung volume, number of receptors and surface area was maintained, which resulted in the inhibition of excessive alveolar differentiation (27). The repair of alveolar epithelium may be promoted thus treating the fibrosis (27). In addition, another study has suggested that nuclear transcription factor activator protein 1 (AP-1) may promote the excessive secretion of type I collagen and due to the antagonism between retinoic acid (RA) and the AP-1 receptor, RA may decrease the activity of AP-1 (28).

In the present study, rats in group W exhibited significantly decreased fibrosis scores, indicated by Masson staining on day 31, when compared with groups M and D. HRCT revealed that only slight fibrotic changes were observed in the middle and late phases; when compared with group D. The Hyp content and serum TGF-β1 concentration in the lung tissues of groups L, W and R were notably decreased. The reasons were presumed as that while RET and ROS elicit receptor interactions through which the cell growth and immune functions were regulated and the expression of TGF-β1 was inhibited, RET receptors may maintain the alveolar volume and quantities and exhibit certain repairing effects, which may inhibit lung fibroblast activation and collagen synthesis.

The present study investigated the effects of ROS and RET in bleomycin-induced pulmonary fibrosis in rats. On day 31 following modeling, the pathological HRCT changes in the lungs of each intervention group were markedly attenuated when compared with group M. Furthermore, the pulmonary fibrosis markers, Hyp and serum TGF-β1, were decreased in the intervention groups when compared with group M. Additionally, during the entire treatment period, the overall conditions, including appetite and weight of the rats were good in group C. However, significant differences in the condition of rats were observed in the treatment groups, which appeared as the early anti-inflammatory effects of ROS and RET showed no significant difference when compared with dexamethasone. The late fibrosis degrees of groups R and W were mild and the mildest degree of fibrosis was observed in group L, which suggested that the treatment groups exhibited an improved condition and the combination of ROS and RET was more effective than the application of a single agent. No significant difference was indicated between ROS and RET.

To conclude, the present findings suggest that ROS and RET have preventive effects against pulmonary fibrosis in rats and the side effects were relatively reduced when compared with glucocorticoids. Whether one or several steps were involved inside the specific anti-fibrotic mechanism still remains to be elucidated. Further investigation is required into the specific mechanisms and targets involved in the inhibition of pulmonary fibrosis, whether these are associated with early anti-inflammatory effects and if these factors may reverse advanced pulmonary fibrosis. Further investigation into the pathogenesis of pulmonary fibrosis may aid the development of novel anti-fibrotic pharmacological agents to treat pulmonary fibrosis.

References

1. Ley B and Collard HR: Epidemiology of idiopathic pulmonary fibrosis. Clin Epidemiol 5: 483-492, 2013.
2. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier JF, Flaherty KR, Lasky JA, *et al*: An official ATS/ERS/JRS/ALAT statement: Idiopathic pulmonary fibrosis: Evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 183: 788-824, 2011.
3. Travis WD, Costabel U, Hansell DM, King TE Jr, Lynch DA, Nicholson AG, Ryerson CJ, Ryu JH, Selman M, Wells AU, *et al*: An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med 188: 733-748, 2013.
4. Moua T, Zamora Martinez AC, Baqir M, Vassallo R, Limper AH and Ryu JH: Predictors of diagnosis and survival in idiopathic pulmonary fibrosis and connective tissue disease-related usual interstitial pneumonia. Respir Res 15: 154, 2014.
5. Rafii R, Juarez MM, Albertson TE and Chan AL: A review of current and novel therapies for idiopathic pulmonary fibrosis. J Thorac Dis 5: 48-73, 2013.
6. Idiopathic Pulmonary Fibrosis Clinical Research Network, Raghu G, Anstrom KJ, King TE Jr, Lasky JA and Martinez FJ: Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. N Engl J Med 366: 1968-1977, 2012.
7. Idiopathic Pulmonary Fibrosis Clinical Research Network; Martinez FJ, de Andrade JA, Anstrom KJ, King TE Jr and Raghu G: Randomized trial of acetylcysteine in idiopathic pulmonary fibrosis. N Engl J Med 370: 2093-2101, 2014.
8. King TE Jr, Bradford WZ, Castro-Bernardini S, Fagan EA, Glaspole I, Glassberg MK, Gorina E, Hopkins PM, Kardatzke D, Lancaster L, *et al*: A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. N Engl J Med 370: 2083-2092, 2014.
9. Calkin AC, Giunti S, Jandeleit-Dahm KA, Allen TJ, Cooper ME and Thomas MC: PPAR-alpha and -gamma agonists attenuate diabetic kidney disease in the apolipoprotein E knockout mouse. Nephrol Dial Transplant 21: 2399-2405, 2006.
10. Iglarz M, Touyz RM, Viel EC, Paradis P, Amiri F, Diep QN and Schiffrin EL: Peroxisome proliferator-activated receptor-alpha and receptor-gamma activators prevent cardiac fibrosis in mineralocorticoid-dependent hypertension. Hypertension 42: 737-743, 2003.
11. Leclercq IA, Sempoux C, Stärkel P and Horsmans Y: Limited therapeutic efficacy of pioglitazone on progression of hepatic fibrosis in rats. Gut 55: 1020-1029, 2006.
12. Kayalar O and Oztay F: Retinoin induced repair in the lung of adult hyperoxic mice, reducing transforming growth factor-β1 (TGF-β1) mediated abnormal alterations. Acta Histochem 116: 810-819, 2014.
13. Davis BH, Kramer RT and Davidson NO: Retinoin modulates rat Ito cell proliferation, collagen, and transforming growth factor beta production. J Clin Invest 86: 2062-2070, 1990.
14. Szapiel SV, Elson NA, Fulmer JD, Hunninghake GW and Crystal RG: Bleomycin-induced interstitial pulmonary disease in the nude, athymic mouse. Am Rev Respir Dis 120: 893-899, 1979.
15. Chua F, Gauldie J and Laurent GJ: Pulmonary fibrosis: Searching for model answers. Am J Respir Cell Mol Biol 33: 9-13, 2005.
16. Limjunyawong N, Mitzner W and Horton MR: A mouse model of chronic idiopathic pulmonary fibrosis. Physiol Rep 2: e00249, 2014.
17. Lee CG, Cho SJ, Kang MJ, Chapoval SP, Lee PJ, Noble PW, Yehualaeshet T, Lu B, Flavell RA, Milbrandt J, *et al*: Early growth response gene 1-mediated apoptosis is essential for transforming growth factor beta1-induced pulmonary fibrosis. J Exp Med 200: 377-389, 2004.
18. Kim KK, Wei Y, Szekeres C, Kugler MC, Wolters PJ, Hill ML, Frank JA, Brumwell AN, Wheeler SE, Kreidberg JA and Chapman HA: Epithelial cell alpha3beta1 integrin links beta-catenin and Smad signaling to promote myofibroblast formation and pulmonary fibrosis. J Clin Invest 119: 213-224, 2009.
19. Asada K, Sasaki S, Suda T, Chida K and Nakamura H: Antiinflammatory roles of peroxisome proliferator-activated receptor gamma in human alveolar macrophages. Am J Respir Crit Care Med 169: 195-200, 2004.
20. Burgess HA, Daugherty LE, Thatcher TH, Lakatos HF, Ray DM, Redonnet M, Phipps RP and Sime PJ: PPARgamma agonists inhibit TGF-beta induced pulmonary myofibroblast differentiation and collagen production: Implications for therapy of lung fibrosis. Am J Physiol Lung Cell Mol Physiol 288: L1146-L1153, 2005.
21. Genovese T, Cuzzocrea S, Di Paola R, Mazzon E, Mastruzzo C, Catalano P, Sortino M, Crimi N, Caputi AP, Thiemermann C and Vancheri C: Effect of rosiglitazone and 15-deoxy-Delta12,14-prostaglandin J2 on bleomycin-induced lung injury. Eur Respir J 25: 225-234, 2005.
22. Milam JE, Keshamouni VG, Phan SH, Hu B, Gangireddy SR, Hogaboam CM, Standiford TJ, Thannickal VJ and Reddy RC: PPAR-gamma agonists inhibit profibrotic phenotypes in human lung fibroblasts and bleomycin-induced pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 294: L891-L901, 2008.
23. Cifelli CJ and Ross AC: All-trans-retinoin distribution and metabolism in vitamin A-marginal rats. Am J Physiol Gastrointest Liver Physiol 291: G195-G202, 2006.
24. Wang L, Potter JJ, Rennie-Tankersley L, Novitskiy G, Sipes J and Mezey E: Effects of retinoic acid on the development of liver fibrosis produced by carbon tetrachloride in mice. Biochim Biophys Acta 1772: 66-71, 2007.
25. Fleissner D, Frede A, Knott M, Knuschke T, Geffers R, Hansen W, Dobos G, Langhorst J, Buer J and Westendorf AM: Generation and function of immunosuppressive human and murine CD8+ T cells by transforming growth factor-β and retinoic acid. Immunology 134: 82-92, 2011.
26. Redlich CA, Delisser HM and Elias JA: Retinoin inhibition of transforming growth factor-beta-induced collagen production by human lung fibroblasts. Am J Respir Cell Mol Biol 12: 287-295, 1995.
27. Massaro GD, Massaro D and Chambon P: Retinoin receptor-alpha regulates pulmonary alveolus formation in mice after, but not during, perinatal period. Am J Physiol Lung Cell Mol Physiol 284: L431-L433, 2003.
28. Schüle R, Rangarajan P, Yang N, Kliewer S, Ransone LJ, Bolado J, Verma IM and Evans RM: Retinoin is a negative regulator of AP-1-responsive genes. Proc Natl Acad Sci USA 88: 6092-6096, 1991.