The effects of Ang-1, IL-8 and TGF-β_1 on the pathogenesis of COPD

JIXU GAO and BINGYAN ZHAN

Department of Respiratory Medicine, Jiaozhou Central Hospital, Qingdao, Shandong, P.R. China

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Abstract. Chronic obstructive pulmonary disease (COPD) is a prevalent smoking-related disease for which no disease-altering therapies currently exist. Airway remodeling is one of the most important mechanisms in the pathogenesis of COPD and is triggered by chronic inflammation mediated by angiopoietin-1 (Ang-1), interleukin-8 (IL-8) and transforming growth factor-β_1 (TGF-β_1). The aim of this study was to investigate the effects of Ang-1, IL-8 and TGF-β_1 on the pathogenesis of COPD. Forty-two COPD patients and 10 healthy adults (group A) were included in this study. We divided the 42 patients into 4 groups (groups B-E) according to the severity of the disease. We investigated the levels of Ang-1, IL-8 and TGF-β_1 and the levels of pulmonary function (PF) in the stable and acute phases of COPD by enzyme-linked immunosorbent assay. We found statistically significant differences in the expression levels of Ang-1, IL-8 and TGF-β_1 between the stable and acute phases in groups B-E. We found statistically significant differences in the expression levels of Ang-1 among all groups in the stable phase. In addition, there were statistically significant differences in the expression levels of TGF-β_1 among all groups. There were statistically significant differences in the expression levels of IL-8 between group A and the other groups in the stable phase. Furthermore, in groups C-E we found higher correlations between Ang-1 and the forced expiratory volume in one second of forced vital capacity (FVC) [FEV_1(%)] and FEV_1/FVC(%) than between TGF-β_1 and FEV_1(%) and FEV_1/FVC(%). We conclude that the blood vessel factor is more closely related to the pathogenesis of COPD.

Correspondence to: Dr Bingyan Zhan, Department of Respiratory Medicine, Jiaozhou Central Hospital, 29th Xuzhou Road, Jiaozhou, Qingdao, Shandong, P.R. China
E-mail: byz96@sina.com

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Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by the chronic obstruction of expiratory flow affecting peripheral airways, associated with pulmonary vascular change, chronic bronchitis (mucus hypersecretion with goblet cell and submucosal gland hyperplasia) and emphysema (destruction of airway parenchyma), together with fibrosis and tissue damage, and inflammation of the small airways. Airway remodeling is one of the most important mechanisms contributing to a reduction in the respiratory airflow in COPD and involves airway and blood vessel factors (1). In the bronchi, epithelial dysregulation results in impaired mucocilliary clearance, overproduction of mucus and squamous cell metaplasia. Other structural changes in COPD, including thickening of the airway wall and reticular basement membrane, have been implicated as factors that contribute to the reduction in airflow (2,3). Apoptosis in human pulmonary artery endothelial cells (HPAECs) in COPD has been revealed to be affected by chronic inflammation involving Ang-1 and TGF-β_1 (4,5).

In COPD, cigarette smoking (CS) may induce airway inflammation. Human cells are characterized by a marked ability to vary their expression levels of interleukin-8 (IL-8), allowing modulation of the concentration of this cytokine to control the degree of neutrophil infiltration in acute exacerbation of COPD (AECOPD). CS has been shown to activate proinflammatory transcription factors to upregulate the expression of IL-8, a proinflammatory mediator associated with COPD (6). Oxidant stress other than hyperoxia is one of the etiological factors for COPD, which has previously been reported to induce IL-8 expression in respiratory epithelial cells. The airway epithelium is one of several sources of IL-8 in the airway and serves as a barrier against invading microorganisms. Airway epithelial release of IL-8 contributes to the host defense by promoting neutrophil chemotaxis and airway inflammation (6). Neutrophils secrete many types of protease that damage the endothelial cells of the airway in AECOPD. A previous study has demonstrated that hypoxia induces the expression of IL-6 and IL-8 in human pulmonary fibroblasts and vascular smooth muscle cells (VSMCs) and stimulates the proliferation of both cell types (7).

TGF-β_1 is a multifunctional cytokine that regulates immune responses, cellular proliferation and differentiation, tissue repair and extracellular matrix production (8). Abnormal TGF-β signaling may explain the increased extracellular

Table I. Correlation of TGF-β_1, Ang-1 and IL-8 levels between all groups in the stable phase (pg/ml).

Group	No.	Ang-1	TGF-β_1	IL-8
A	10	1313.76±185.1	14.31±1.05	4.55±0.36
B	10	2046.36±131.25[a]	43.22±5.15[a]	5.79±0.55[a]
C	11	1574.87±143[ab]	31.97±3.4[ab]	5.94±0.59[a]
D	11	1021.37±79.75[a-c]	27.52±1.34[a-c]	6.06±0.52[a]
E	10	787.94±77.09[a-d]	26.25±0.88[a-c]	6.52±0.32[a-d]

Ang-1, angiopoietin-1; TGF-β1, transforming growth factor-β1; IL-8, interleukin-8. [a]P<0.05 compared with group A. [b]P<0.05 compared with group B. [c]P<0.05 compared with group C. [d]P<0.05 compared with group D.

matrix observed in the distal airways of patients with mild or severe COPD, which may compromise repair in the airspace compartment, leading to histologic emphysema (9). TGF-β_1 has been reported to protect HPAECs against apoptosis (4).

Angiogenesis, a complex process whereby blood vessels sprout from extant microvasculature, involves the coordination of multiple events, including degradation of the basement membrane by proteases, proliferation and migration of endothelial cells, lumen formation, basement membrane reassembling, recruitment of pericyte and/or vascular smooth muscle cells (SMCs), vascular maturation and, ultimately, blood flow (10,11). Angiopoietin-1 (Ang-1), a ligand for the endothelial cell-specific Tie2 receptors, promotes the migration and proliferation of endothelial cells. Evidence for increased vessel survival in response to Ang-1 *in vivo* is provided in studies of radiation-exposed mice, in which endothelial survival in the microvessels of the airway is increased by the ligand (12). However, how the two factors function in COPD of varying severity has been little reported. In the present study, our aim was to investigate the three cytokines in different COPD patient groups and to correlate these levels with the degree of COPD severity.

Materials and methods

A case control study was conducted and all subjects were randomly selected by the Department of Respiratory Medicine, Jiaozhou Central Hospital, Qingdao. We assessed a consecutive convenience sample of 42 male patients with a diagnosis of COPD (GOLD stages I-IV). A total of 42 subjects with COPD and 10 without clinical or functional signs of COPD (controls) were included in the study. The 10 control subjects were non-smokers. We divided the COPD patients into 4 groups (B, C, D and E) according to the severity of the COPD (2010 GOLD). Exclusion criteria were: presence of a motor or neurological disorder, indication and/or use of long-term home oxygen therapy, pulmonary rehabilitation in the previous year, concomitant diagnosis of malignant disease, chronic heart failure and liver disease or nephropathy. The study was approved by the ethics committee of Jiaozhou Central Hospital. Patients provided informed consent.

Pulmonary function (PF) tests. Spirometry pre- and post-administration of a bronchodilator (400 μg salbutamol via inhalation dosimeter) was performed in the morning on patients with an empty stomach in order to test the forced vital capacity (FVC, L) and the forced expiratory volume in one second of FVC (FEV_1, L), and the FEV_1/FVC ratio was determined.

Sample collection. A 3-ml sample of blood was obtained from every subject in the acute exacerbation and stable phases of COPD using a hemospast. Sampling took place in the morning from patients having an empty stomach. All samples were centrifuged (3000 rpm) to provide 1 ml serum from every sample which was stored in liquid nitrogen. Plasma samples were stored as individual aliquots at -80˚C until use. The levels of IL-8, TGF-β_1 and Ang-1 were measured in the serum using kits from R&D Systems (Minneapolis, MN, USA) for measuring human IL-8, TGF-β_1 and Ang-1.

Statistical analysis. Data were analyzed using SPSS 11.5 software. Descriptive data for continuous variables with a normal distribution are presented as the mean ± standard deviation (SD) or median and range for data not normally distributed. Mean square error analysis was used for all groups. The probability of error (P-value) was calculated. P<0.05 was considered to indicate a statistically significant result. The correlation coefficient (R-value) was calculated and significant differences between the levels of cytokines (TGF-β_1, Ang-1) and PF (FEV_1, FVC) within each group were identified.

Results

Highly significant differences in the levels of Ang-1 and TGF-β_1 among all groups. The levels of TGF-β_1 in group B were higher than those in group A. There was a significant difference in the levels of TGF-β_1 between groups C and D but no significant difference in the levels of TGF-β_1 between groups D and E. There were significant differences between the Ang-1 levels in group A and those of groups B-E. There was no significant difference in the levels of IL-8 between groups B and C. The levels of IL-8 in group E were higher than those in the other groups (P<0.05; Table I).

Highly significant differences in the levels of Ang-1, IL-8 and TGF-β_1 among all groups. For all groups, the levels of Ang-1 in the stable phase were significantly higher than those in the acute exacerbation phase. The levels of IL-8 in the acute

Table II. Correlation of TGF-β_1, Ang-1 and IL-8 levels between all groups in the stable and acute exacerbation phases (pg/ml).

Group	No.	Ang-1		TGF-β_1		IL-8	
		AE	SP	AE	SP	AE	SP
B	10	1779.34±109.27	2046.36±131.2[a]	48.84±4.57	43.22±5.15[a]	7.29±0.64	5.79±0.55[a]
C	11	1459.05±111.66	1574.87±143[a]	37.02±3.64	31.97±3.4[a]	6.93±0.45	5.94±0.59[a]
D	11	873.99±67.72	1021.37±79.75[a]	34.75±1.45	27.52±1.34[a]	7.05±0.47	6.06±0.52[a]
E	10	668.98±82.33	787.94±77.09[a]	34.68±2.81	26.25±0.88[a]	7.39±0.30	6.52±0.32[a]

[a]P<0.05 compared with AE. Ang-1, angiopoietin-1; TGF-β_1, transforming growth factor-β_1; IL-8, interleukin-8; AE, acute exacerbation phase; SP, stable phase.

Table III. Correlation of PF levels between all groups in the stable phase.

Group	No.	FEV_1(%)	FEV_1/FVC(%)
A	10	100±7	85.13±4.52
B	10	85.6±2.36[a]	65.5±0.21[a]
C	11	63.9±5.82[a,b]	58.7±3.51[a,b]
D	11	41.7±2.59[a-c]	58.3±2.76[a,b]
E	10	28.89±2.06[a-d]	58.48±2.86[a-c]

[a]P<0.05 compared with group A. [b]P<0.05 compared with group B. [c]P<0.05 compared with group C. [d]P<0.05 compared with group D. PF, pulmonary function; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second of FVC.

Table IV. R-values between TGF-β_1, Ang-1 and PF.

		Factor	
Group	PF	Ang-1	TGF-β_1
A	FEV_1(%)	0.005[a]	0
	FEV_1/FVC(%)	0.055[a]	0.008[a]
B	FEV_1(%)	0.007[a]	0.01[a]
	FEV_1/FVC(%)	0.184[a]	0.048[a]
C	FEV_1(%)	0.877[a]	0.004[a]
	FEV_1/FVC(%)	0.475[a]	0.104[a]
D	FEV_1(%)	0.76[a]	0.146[a]
	FEV_1/FVC(%)	0.519[a]	0.005[a]
E	FEV_1(%)	0.618[a]	0
	FEV_1/FVC(%)	0.608[a]	0

[a]P<0.05. PF, pulmonary function; Ang-1, angiopoietin-1; TGF-β_1, transforming growth factor-β_1. FVC, forced vital capacity; FEV_1, forced expiratory volume in one second of FVC.

exacerbation phase were significantly higher than those in the stable phase for each group. The levels of TGF-β_1 in the stable phase were significantly lower than those in the acute exacerbation phase for all groups (Table II).

Highly significant differences in the levels of FEV_1 among all groups. There were significant differences in the levels of FEV_1/FVC among all groups with the exception of C and D (Table III).

Positive significant correlations between Ang-1 and TGF-β_1. We found positive significant correlations between Ang-1 and TGF-β_1 levels for each stage of COPD according to its FEV_1 and FEV_1/FVC values (P<0.05; Table IV).

Discussion

It is generally accepted that CS is the most important risk factor for COPD. A difference has always existed between the prevalence of COPD in males and females (13). We selected only male patients in order to avoid gender error. There was no significant difference in the age levels among the groups. This trial also ruled out various conditions, including malignant neoplasm and severe myocardial remodeling, in order to exclude the effects of these diseases on cytokines, and pulmonary rehabilitation, which may improve airway function, to provide objective results.

IL-8 has been implicated in a number of inflammatory diseases, including adult respiratory distress syndrome (ARDS) (14) and COPD (15). IL-8 is produced by a number of cell populations, including activated bronchial epithelial cells, macrophages and neutrophils, and may be an initiating agent for cell migration in COPD CS. It is a potent chemoattractant for neutrophils and monocytes and, depending upon the anatomical location, may contribute to the mobilization of these leukocyte populations into the lung. In some studies, the levels of IL-8 have been found to be increased in the induced sputum of patients with COPD and, unsurprisingly, the levels appear to correlate with the proportion of neutrophils (16) and are also increased in the sputum during exacerbations (17). Neutrophils release neutrophil elastase (NE) to damage the airway, which induces fibroblasts to release TGF-β_1 to repair the airway and leads to epithelial thickening and decreased PF (8). Neutrophils decrease in the stable phase of COPD. With the exacerbation of COPD, increased numbers of inflammatory cells, including lymphocytes, neutrophils, eosinophils and mast cells, and mediators, including IL-8, are implicated in the airway inflammation in the stable phase of COPD (18).

Through the trial we discovered that there were no significant differences in the levels of IL-8 among the groups B, C and D; the levels of IL-8 in these groups are higher than those of group A in the stable phase. Moreover, the levels of IL-8 in group E are higher than those in the other groups in the stable phase. We revealed that the infiltration of phlogocytes from the airway in the stable phase of COPD may be persistent. Ang-1 has been reported to induce significant increases in IL-8 production in pulmonary artery endothelial cells (ECs) through the induction of transcription and enhanced IL-8 mRNA stability (19). Furthermore, the levels of IL-8 in the acute exacerbation phase were higher than those in the stable phase. From group B to E, the levels of Ang-1 decrease in turn. The previously discussed factors may contribute to these results. Moreover, elevated IL-8 levels in COPD may be an initiating factor of the airway and blood vessel remodeling processes.

Tissue injury and inflammation are associated with remodeling as observed in several airway diseases, including asthma and COPD (20). One feature of airway remodeling is smooth muscle cell hyperplasia, which impacts airway caliber and decreases lung function (21,22). The small airway epithelium may play various important roles in COPD pathophysiology. Firstly, increased epithelial thickness, which contributes to airway wall thickness and reduced airway radius, is associated with airflow limitation in COPD subjects (21). Secondly, a study (23) has suggested that the squamous airway epithelium promotes peribronchiolar fibrosis via the increased secretion of interleukin-1β, resulting in increased TGF-β_1 secretion and fibrosis. Thirdly, the secretion of epithelial mucins from hyperplastic goblet cells is likely to contribute to lumen obstruction (24). Finally, the small airway epithelium may promote the recruitment of neutrophils, macrophages and T lymphocytes via the secretion of specific chemoattractants. Apoptosis, programmed cell death, is a major mechanism by which cells are removed from tissues. Apoptosis may be influenced by a variety of inflammatory cytokines, including Ang-1 and TGF-β_1, in HPAEC in COPD.

An active and complex remodeling process is present in the peripheral lung when COPD develops, resulting in small airway fibrosis and a variable degree of emphysema. Fibroblasts are the primary cell type responsible for the production and maintenance of the extracellular matrix (25). Alterations in fibroblast function may therefore be significant in COPD. The pleiotropic cytokine, TGF-β, has distinct effects on homeostasis and repair mechanisms (26). Genetic association studies of patients with emphysema and histologic surveys of lungs from patients with COPD of varying severity have implicated disturbances in TGF-β signaling as important components of disease pathogenesis (27). The levels of TGF-β_1 in group B were higher than those of group A in the stable phase ($P<0.05$). In the prophase of COPD, hyperplasic fibroblasts express TGF-β_1. While increased TGF-β_1 signaling may explain the increased extracellular matrix observed in the distal airways of patients with severe COPD, reduced signaling with suboptimal matrix deposition may compromise repair in the airspace compartment, leading to histologic emphysema. It is considered that TGF-β modulate airway functions and regulates blood vessel functions in COPD (28). We propose that during COPD pathogenesis, irrespective of GOLD stage, fibroblasts from the peripheral lung are promoted to repair tissue damage, but this repair response becomes insufficient in the more advanced stages of the disease. The levels of TGF-β_1 in group C were lower than those of group B in the stable phase and there was no significant difference in the levels of TGF-β_1 between groups D and E ($P>0.05$). In addition, the levels of TGF-β_1 in group E were higher than those of group A. With the alleviation of the chemotactic response of fibroblasts and the gradual occurrence of inogenesis, the levels of TGF-β_1 decreased gradually. A previous study has suggested that TGF-β_1 protects against apoptosis mediated through mitochondrial dysfunction (29). This theory was further supported by the observations that TGF-β_1 prevented the reduction of Bcl-2 induced by serum deprivation and the blockade of VEGF receptors in COPD. In the prophase of COPD, the higher levels of TGF-β_1 may protect HPAECs against apoptosis, whereas with severe COPD, decreased levels of TGF-β_1 may cause apoptosis of HPAECs.

The vascular wall is mainly composed of ECs and SMCs. The crosstalk between these two cell types is critical to the vascular maturation process. Genetic studies suggest that the Tie2/Ang-1 pathway regulates vascular remodeling. TGF-β_1 has been reported to negatively regulate Ang-1 expression induced by the platelet-derived growth factor-B (PDGF-B) in SMCs (30).

COPD patients have a significantly reduced capillary length and length density (34). Vascular regression is another facet of lost vascular homeostasis, which is ultimately involved with the lung parenchyma and muscle loss observed in emphysema patients (31). The effects of Ang-1 on ECs and blood vessels broadly fall into two categories: those associated with the promotion of vessel protection, and those related to vessel remodeling and angiogenesis. One study reports that Ang-1 exerts pro-survival activity on neutrophils, which is mainly driven through IL-8 release. Blocking IL-8 and the IL-8 receptor CXCR2 significantly inhibited angiogenesis (32). Ang-1 inhibits apoptosis and inflammatory responses and promotes differentiation, sprouting and migration. The mean value of Ang-1 was found to differ significantly among all groups. The levels of Ang-1 in group B were higher than those in group A. It is likely that early in the development of COPD there are directly toxic effects of cigarette smoke on the lung vessels, and in the later stages of COPD, hypoxia-induced lung vessel remodeling may occur. Moreover, the protective effect of TGF-β_1 against apoptosis is dependent upon the mechanism of stimulation. The higher levels of TGF-β_1 and the directly toxic effects of cigarette smoke may lead to the high levels of Ang-1 in group B. The levels of Ang-1 in groups C-E were lower than those of group B in the stable phase. The R-value between Ang-1 and FEV_1 was higher than that between TGF-β_1 and FEV_1, which suggests that loss of capillaries may contribute to the severe airflow obstruction. The loss of microvessels may also be a cause of the muscle-wasting component of end-stage COPD. It has been shown that a consequence of lung cell apoptosis is the failure of angiogenic repair due to recurrent attacks, which may support these results. The levels of Ang-1 in the acute exacerbation phase were lower than those in the stable phase. Therefore, the levels of Ang-1 in the AECOPD patients may have some relationship with the repeated airway infection, leading to reduced capillary length

and length density. The levels of Ang-1 in group D were lower than those in group A (normal control) in the stable phase ($P<0.05$). Severe COPD reduces the number of capillaries in the stable phase, which may contribute to severe pulmonary hypertension. Ang-2 is an Ang-1 antagonist that is involved in vascular destabilization and remodeling. Serum Ang-2 levels are significantly elevated during acute exacerbations of COPD, as compared with stable COPD (33), which supports the results of the current trial.

We conclude that airway and blood vessel factors interact and mutually promote each other and, during this process, the blood vessels may contribute to airway remodeling. A more detailed expression analysis will be useful for further understanding the complex pathology of COPD.

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