

TUESDAY, SEPTEMBER 5TH 2006

## 392. Pulmonary fibrosis: pathophysiology and therapeutic approaches

4403

### Effects of aerosolized earthworm fibrinolytic enzyme on bleomycin-induced pulmonary fibrosis in rats

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**Objective** There is increasing evidence that the unbalance between coagulation and fibrinolysis plays a central role in the development of pulmonary fibrosis associated with lung injury. Earthworm fibrinolytic enzyme(EFE) was proved to have functions decreasing fibrinogen and fibrin as well as activating fibrinolytic system. The aim of this study is to investigate the effects of aerosolized EFE on bleomycin-induced pulmonary fibrosis in rats.

**Methods** 72 SD male rats were divided randomly into three groups, the Bleomycin(BLM) group receiving intratracheally BLM (5mg/kg) solution 0.2~0.3ml, control group receiving the same amount of normal saline, the EFE group receiving EFE (2500IU/kg) by aerosolization once daily after BLM instillation. Lung histopathology, immunohistochemistry for TGF- $\beta_1$ , lung hydroxyproline, the levels of the tissue plasminogen activator(tPA), urokinase PA(uPA), and PA inhibitor(PAI) in blood and lung were observed by 7, 14 and 28 day of experiment, respectively.

**Results** The pulmonary fibrosis were significantly improved, the TGF- $\beta_1$  expression and hydroxyproline content of lung tissue decreased ( $p < 0.01$ ) in EFE group compared with BLM group. Consistently, the tPA levels of lung and plasma were increased and the PAI levels decreased ( $p < 0.01$ ) in EFE group. The uPA levels were increased but without significant difference. There were no significant difference in prothrombin time and APTT among the groups.

**Conclusions** Earthworm fibrinolytic enzyme can decrease bleomycin induced pulmonary fibrosis and TGF- $\beta_1$  expression while increasing fibrinolytic activation. It is suggested that fibrinolytic strategies may be useful for the therapy of fibrotic lung diseases.

4404

### Effects of bone marrow mesenchymal stem cells on bleomycin induced pulmonary fibrosis in rats

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**Objective** To study the effects of bone marrow mesenchymal stem cells (MSCs) on pulmonary fibrosis.

**Methods** The bone marrow MSCs were harvested from 6 weeks old male SD rat. Pulmonary fibrosis models were made by intratracheal instillation of Bleomycin(BLM) (5mg/kg in 0.3ml normal saline) to female SD rats. On the first and seventh day after receiving BLM, Group 1 and 2 were administered male MSCs via vena caudalis, their respective control, group 3 and 4 were administered the same amount of PBS. Group 5 is BLM induced pulmonary fibrosis model without intervenient treatment, group 6 is normal control receiving intratracheal instillation of NS alone. The pathologic changes and hydroxyproline contents of lung tissue were investigated while the rats were killed by the 28 day of experiment. The sry gene of Y chromosome was detected by PCR.

**Results** The fibrotic changes and hydroxyproline contents of lung tissue were decreased significantly in MSCs intervenient rats(G1 & G2) compared with non MSCs intervenient(G3 & G4). Early administration of MSCs(G1) resulted in more improvement than later use of MSCs(G2). sry gene (322bp) was detected in lungs of female rats receiving MSCs in the first day of BLM induced lung injury.

**Conclusions** MSCs may be involved in the lung injury and repair process, especially in the early stage. MSCs can shift to lung, decrease lung injury and fibrosis, promote lung repair although the exact mechanism is not clear. MSCs may have potential therapeutic role for fibrotic lung disease.

4405

### Refractory remodeling of lymphatics with rare lymphangiogenesis as a cause of severity of IPF

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The pivotal role of lymphatic drainage for alveolar clearance is well known in pulmonary edema, but not interstitial pneumonias. Because the superficial lymphatics in the lungs are distributed in the subpleural space and interlobular septa, we hypothesized that lymphatic impairment would occur in the lungs of idiopathic pulmonary fibrosis (IPF) and increase its severity. We compared the remodeling of superficial lymphatics among the biopsied and autopsied lungs of patients with interstitial pneumonias, including IPF, organizing pneumonia (OP), and non-specific interstitial pneumonia (NSIP). Morphometric analysis clearly revealed an extreme decrease of the lymphatics in the interlobular and subpleural space in which massive fibrosis increased without development of new lymphatics in the lungs of patients with IPF. In contrast, in the lungs of patients with OP and cellular NSIP, abundant new lymphatics had extended into the alveolar lesions from the superficial lymphatics, especially into granulation tissues within the alveolar space. The expression of lymphatic growth factors, both VEGF-C and D, were augmented in the lungs of all the interstitial pneumonias examined, but the massive fibrotic tissues developed in IPF, including fibroblastic foci, blocked lymphatics from the cells producing VEGF-C and/or D. These results suggest the disruption of the superficial lymphatics with rare lymphangiogenesis would impair alveolar clearance, delay organ repair and cause severe disease progress of patients with IPF.

4406

### Noninvasive positive pressure ventilation in patients with acute exacerbation of idiopathic pulmonary fibrosis

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**Background** Effectiveness of NPPV has been demonstrated in immunosuppressed patients with acute respiratory failure. The outcome of acute exacerbation (AE) of IPF was reported to be very poor. Because patients with AE of IPF tend to be treated with immunosuppressive therapy, we suppose that NPPV may improve survival.

**Purpose** We evaluated the effectiveness of NPPV for the treatment of AE of IPF. **Methods** Patients who fulfilled the criteria of AE of IPF were included in the study. The definition of AE of IPF is as follows; During chronic course of IPF, 1) acute worsening of dyspnea within a month, 2) bibasilar honeycombing with newly developing ground glass attenuation and/or consolidation on HRCT scans, 3) deterioration of PaO<sub>2</sub> more than 10mmHg under same condition, 4) exclusion of other known causes of exacerbation, such as pulmonary infection, pneumothorax, and heart failure. Survival after 3 months was evaluated. All patients were treated by BiPAP Vision(Respironics Inc, Murrysville, PA, USA).

**Results** This study included 11 patients (age 72.3 $\pm$ 7.7 y.o., male/female 7/4, PaO<sub>2</sub>/FiO<sub>2</sub> 141.4 $\pm$ 53.5, APACHE II score 14.2 $\pm$ 5.5). All patients were treated with steroid pulse therapy, followed by combination of corticosteroid and immunosuppressant. Length of NPPV was 5.4 $\pm$ 3.8 days. Five of 11(45%) patients avoided intubation and survived after 3 months. Four of 6 patients who failed NPPV required intubation, and the other 2 patients refused endotracheal intubation. All 6 patients who failed NPPV died within 3 months.

**Conclusion** Our findings indicate that NPPV is a viable option for the management of acute respiratory failure in patients with acute exacerbation of IPF.

4407

### Induction of TGF- $\beta$ and reactive oxygen species expression in bronchial epithelial cell by urban ambient air particulate matter

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Ambient air particulate matter  $\leq 10 \mu\text{m}$  in aerodynamic size (PM) is a complex mixture of organic and inorganic compounds. PM can involve to lower respiratory tract because it's size is small. PM has received increasing attention because of its role in adverse respiratory health effects-especially acute inflammation, yet little is known regarding the fibrotic effects of PM on pulmonary cell types. We investigated TGF- $\beta$ 1 and ROS produced by normal epithelial cell line exposed to particles collected from Incheon city. Each was compared with those in the control that non-exposed cells to the particle. We collected air samples using high volume air sampler (Sibata Model HV500F) with airflow at 500 L/min for at least 6hrs. We collected particulate matter in aerodynamic size  $\leq 10 \mu\text{m}$ . Bronchial epithelial cells were exposed to 10-500  $\mu\text{g/ml}$  of a suspension of PM for 24-48hrs. TGF- $\beta$ 1 was detected by western blotting. After incubation with DCF-DA at each cells, ROS was detected by measurement of DCF using FACScan. We also measured that ROS and TGF- $\beta$  after prepared N-Acetyl-Cysteine (NAC) 5mM. TGF- $\beta$  expression was high after exposed to PM compared to the control at 24hrs. ROS of cells after

TUESDAY, SEPTEMBER 5TH 2006

exposed to PM was significantly higher than non-exposed control at 24, 48hrs. (at 24hrs: 10, 50, 100, 250, 500  $\mu\text{g}/\text{ml}$  of PM10 vs control, at 48hrs: 10, 100, 250  $\mu\text{g}/\text{ml}$  of PM10 vs control,  $p < 0.05$ ). After NAC, ROS and TGF- $\beta$  exposed to PM has significantly decreased compare to non-NAC. These results suggest that PM10 has fibrotic effect in bronchial epithelial cells via ROS induction.

**4408****Centrilobular fibrosis (CLF): a distinct histological pattern in systemic sclerosis**

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A new histological pattern of idiopathic interstitial pneumonia characterized by an aggressive bronchocentric scarring with derangement of lobular architecture, basophilic intraluminal contents was previously described by our group as Centrolobular Fibrosis (CLF) (Pathol Res Pract 198: 577-583, 2002). Since chronic aspiration by gastroesophageal reflux has been suggested as the main pathophysiological mechanism for this severe form of lung involvement, our aim was to verify if CLF is found in Systemic Sclerosis (SSc).

**Material and Methods:** Twenty-eight consecutive open lung biopsies of patients with SSc were studied. A chest radiologist unaware of the clinical/histological diagnosis systematically evaluated all HRCT.

**Results:** The most frequent histological subtype found was CLF (35.7%), followed by Bronchocentric Cellular Interstitial Pneumonitis associated with non-specific interstitial pneumonia (BCIP/NSIP) in 32.15% and NSIP (18%). Only two patients (7.15%) were classified as UIP. We also found 2 isolated cases of Pulmonary Hypertension (PH) and RB-ILD. All patients had a restrictive patterns and their %FVC were: CLF  $62.2 \pm 13.41$ ; BCIP/NSIP  $63.3 \pm 13.9$  and NSIP  $72.2 \pm 9.17$ ;  $p > 0.05$ . The final histological presumptive diagnosis was correct in 7 (70%) of CLF patients by HRCT and 3(30%) were incorrectly referred as UIP or NSIP. Almost 80% of the BCIP/NSIP patients were misdiagnosed as NSIP by the radiologist and the "pure" NSIP patients were correctly referred by HRCT in 80% of the cases.

**Conclusion:** This is the first report of centrilobular fibrosis in SSc and the identification of this subgroup of patients will certainly contribute for a more appropriate therapeutic approach.

**4409****Effect of ambulatory oxygen on walking distance in normoxic pulmonary fibrosis**

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Domiciliary oxygen, including ambulatory strollers, is seldom offered to patients with pulmonary fibrosis normoxic at rest, who often desaturate severely during exercise. We evaluated the effect of ambulatory oxygen in 8 patients with pulmonary fibrosis (5 UIP, 1 secondary to sarcoidosis, 1 to allergic extrinsic alveolitis, and 1 radiation-induced), with mean age  $62 \pm 1$  years (M  $\pm$  SE) and VC  $41 \pm 7\%$  of predicted, who were normoxic at rest (PaO<sub>2</sub>  $61 \pm 1$  mmHg, Hb saturation  $93 \pm 0.2\%$ ) but presented oxygen desaturation ( $< 88\%$  for more than 2 minutes) in a preliminary 6-minute walking test. After assessing the oxygen flow to correct exercise-induced hypoxemia (Guyatt, AJRCCM 2001, 163:942), each patient underwent two separate walking tests, according to a double-blind randomized cross-over design, while breathing either oxygen or air, delivered by nasal cannulas at the same flow. Outcomes included distance walked, oxygen saturation, and visual analogue scales for dyspnea and preferences among treatments. Distance walked during placebo ( $149 \pm 26$  m) was markedly smaller than during oxygen ( $291 \pm 21$  m,  $p < 0.005$ ). Dyspnea at the end of test was also greater after placebo ( $6.3 \pm 0.7$ ) than after oxygen ( $4.1 \pm 0.7$ ,  $p < 0.05$ ). Oxygen saturation at the end of test was  $83 \pm 1\%$  after air and  $91 \pm 1\%$  after oxygen ( $p < 0.001$ ). All the patients expressed a preference toward oxygen when compared both to air ( $8.2 \pm 0.3$ , out of a maximum of 10) or to nothing ( $7.1 \pm 0.5$ ). We are not aware of any other current treatment able to provide a similar benefit to patient with pulmonary fibrosis, and we urge that all these patients should be tested for exercise-induced desaturation and offered a practical form of ambulatory oxygen.

**4410****Efficacy and safety of etanercept in patients with idiopathic pulmonary fibrosis (IPF)**

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Efficacy and safety of etanercept (ETN) was evaluated a 48-wk, double-blind trial in 87 pts with progressive IPF (FVC  $\geq 45\%$  predicted, D<sub>1</sub>CO<sub>c</sub>  $\geq 25\%$  predicted, PaO<sub>2</sub>  $\geq 55$  mmHg, or O<sub>2</sub> saturation  $\geq 88\%$  on room air at rest). Pts received ETN (25 mg SC) or placebo (P) twice wkly. 1° endpoints were change from baseline to Wk 48 (LOCF) on FVC% predicted, D<sub>1</sub>CO<sub>c</sub>% predicted, and A-aO<sub>2</sub> gradient

(at rest). No significant difference in baseline lung function was observed between groups. However, at 48 wks, pts receiving ETN showed a trend toward reduced disease progression in 1° and 2° endpoints (p ns).

Change from Baseline at Wk 48, % (SD)

	ETN (n=45)	Placebo (n=40)	Adjusted Treatment Difference, Mean (SE)	P value
<b>1° Endpoints</b>				
FVC (% Predicted)	-2.5 (8.9)	-5.4 (7.1)	-2.9 (1.8)	0.1044
DLCOC (% Predicted)	-2.4 (6.6)	-4.8 (8.2)	-2.2 (1.6)	0.1556
P (A-aO <sub>2</sub> ) Difference (mmHg)*	2.8 (12.2)	1.7 (11.5)	0.6 (2.5)	0.8001
<b>2° Endpoints</b>				
Total Lung Capacity (% Predicted)	-1.9 (6.4)	-2.7 (8.1)	-1.0 (1.5)	0.5157
Resting O <sub>2</sub> Saturation (%)	-0.4 (4.1)	-1.8 (4.1)	-1.3 (0.9)	0.1377
6 Min Walk Distance (m)	0.2 (95.8)	-14.7 (112.5)	-14.8 (21.5)	0.4936

\*N=44 for ETN

In a post-hoc analysis, 15 (33%) and 22 (55%) pts died or had disease progression ( $\geq 10\%$  decline in FVC [L]) at last visit, in the ETN and P groups, respectively ( $p=0.052$ ). There was no significant between-group difference in progression between baseline and 48 wks by HRCT. There were no significant between-group differences in AEs, serious AEs, infections, or serious infections. Although not statistically significant, ETN therapy showed a trend toward reduced disease progression, walk distance, and QOL in pts with progressive IPF.