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Patterns of cytokine release and evolution of remote organs from proximal femur fracture in COPD rats

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ABSTRACT

Background: Chronic obstructive pulmonary disease (COPD) is at increased risk for developing osteoporosis (OP) with subsequent proximal femur fracture. The presence of COPD is suggested to be a strong risk factor for proximal femur fracture or hip fracture. However, what happen behind it is not clearly understood.

Objective: To investigate the pattern of cytokine (TNF-a, IL-6, and IL-10) releases in pulmonary and hepatic in rats with COPD suffering from proximal femur fracture, and its possible adverse effect on pulmonary and hepatic.

Methods and subjective: This paper has two parts. In the first part, we describe the procedure of COPD model in detail. In the second part, we study the influences of fracture on the COPD rats. 5 months WISTAR rats with 37 weeks cigarette smoking exposure (CS group) were dynamically determined for pulmonary function, inflammatory response in bronchoalveolar lavage fluid (BALF), histological changes in pulmonary in the first part. When the COPD model is proved to be successful, we begin the second part. COPD rats were euthanized at 2, 24, 48, 72, and 96 h after proximal femur fracture (fracture group) or anaesthesia (control group). Cytokines (TNF-a, IL-6, and IL-10) and myeloperoxidase activity of pulmonary and hepatic (MPO) were measured with enzyme-liked immunosorbent assay technique. Permeability changes of the lung were assessed via bronchoalveolar lavage, and those of the liver via assessment of oedema formation. Tissues were further examined microscopically.

Results: The current sidestream cigarette smoke induced rat COPD model has been proved an adequate animal model with several advantages as assessed by dynamically monitored lung mechanics and pathological changes for 37 weeks. In the second part, TNF-a, IL-6, and IL-10 levels of pulmonary tissue were significantly increased after proximal femur fracture compared to control rats. TNF-a, and IL-6 levels in pulmonary peaked at 2 h, 24 h in fracture group, whereas IL-10 level peaked at 24 h and 96 h. Pulmonary myeloperoxidase activity, permeability and histological score in fracture group were remarkably elevated, and peaked at 24 h. In addition to TNF-a, all above parameters did not return to normal through our study. Hepatic in COPD rats showed notable increase of cytokines (TNF-a, IL-6, and IL-10), myeloperoxidase activity, histological score, and permeability in fracture group compared to control rats, and severity of these changes were much lower than in pulmonary. Apart from TNF-a, the peak of these parameters was at 24 h after fracture. Changes of cytokines, MPO activity, permeability and histological score in pulmonary and hepatic in control rat were little changed.

Conclusion: COPD rats produced a remarkably increase of inflammatory response (TNF-a, IL-6, IL-10) in lung (liver) after proximal femur fracture, which lead to lung (liver) injury, as evidence by changes of MPO, permeability, and histological scores in local organs.

Introduction

Chronic obstructive pulmonary disease (COPD) refers to a group of diseases (including chronic bronchitis and emphysema) that are characterised by airflow limitation that is progressive in nature and not fully reversible, mainly smoking-related.[15] Certain therapies[30] used in COPD, such as oral and inhaled corticosteroids, increases the risk of osteoporosis, and make elderly more frail to bone fracture, such as proximal femur fracture or hip fracture. Persons with COPD have a 60–70% higher risk of death following proximal femur fracture than those without, and increases 1-year mortality[3,5,4] times as that of persons without bone fracture.[6] What damage is inflicted on to elderly patients with COPD in case

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of proximal femur fracture? We fail to locate any publications in the electronic literature search through system or systems that shed light on the issue.

Recently, numerous studies[18,22] focus on normal trauma have shown that multiple trauma is associated with the development of systemic inflammatory response syndrome and strongly correlated with Multiple Organ Dysfunction Syndrome (MODS). Lung is the first and primary target organ to be affected in the post-injury period, and acute lung injury increases the incidence and mortality from multiple-organ failure.[19,27] The question remain, what is known about changes in immune reactivity in the traumatized elderly patient with COPD? This study is designed to determine the changes in variables of inflammation under these conditions.

Materials and methods

This paper consists of two parts. In the first part, we describe how we make the model of COPD in detail. In the second part, we studied the influences of fracture on the COPD rats.

First part

Animal care

5 months old male Wistar rats, which equals 1/4 of their life expenditure of 21 months (purchased from Xing Wang farm, Beijing, and quarantined for 1 week before exposure to tobacco smoke.), weighing 600–700 g, were housed under pathogen-free conditions and kept at 25 °C with 12-h light or dark cycles. These models really mimic a person start smoking at 19-year old (suppose life expenditure for a person is 80 years old). The animals were caged in groups of five. After 1 week of conditioning, rats were randomly divided into Sham group (clean air exposed only) and cigarette smoke exposed groups (CS group). The rats had free access to water and chew (laboratory animal feed, Beijing HFK Bio-Technology). All rats were maintained according to international guidelines on the ethical use of animals and were approved by Beijing Army General Hospital.

Smoke apparatus

The exposure system used was a modification according to system Zheng described.[33] The smoke apparatus consisted of four major parts including a cigarette burner box, two circulation fans and an inhalation chamber. The cigarette burner box had a slide door for controlling air supply and handling cigarette. The big circulation fan is connected to the cigarette burner box and blown sidestream cigarette smoke into the inhalation chamber sized 80 cm (length) × 50 cm (width) × 50 cm (height). The chamber had enough room for a group of ten rats. The rats were placed in a chamber through the side door. In order to make the smoke uniform, a small circulation fan was fixed on the roof of the chamber. There was an air control hole at the roof of the chamber. The smoke concentration in the exposure chamber was controlled by their control hole and cigarette burning speed. The concentration of the sidestream cigarette smoke aerosol in the chamber could be kept almost constant during exposure. In order to observe rats during smoke treatment, two glass windows were installed on both sides of the chamber.

Tobacco smoke exposure

A group of ten rats were placed in the inhalation chamber. Once these rats were settled, the sidestream cigarette smoke was consistently delivered into the chamber by passing air at a rate of approximately 15 min per three cigarettes. Animals were exposed for 1 h/day for the first 7 days, 2 h/day for a total of 36 weeks. Commercial filtered cigarettes (trade name, Zhong Nan Hai from the Beijing cigarette Factory, China) containing 14 mg tar (equivalent to 1.5-fold of tar quantity in the Kentuchy Reference Cigarette 2R4F) and 1.4 mg nicotine (equivalent to 1.4-fold of nicotine quantity in the Kentuchy Reference Cigarette 2R4F) per cigarette were used in our study.

Evaluation of the model of COPD

In order to determine the success of COPD model, we need to evaluate respiratory function, histological structure of rat lungs, and cytokine in Bronchoalveolar lavage fluid (BALF). At the end of 4 weeks, 8 weeks, 12 weeks, 24 weeks, 37 weeks of smoke exposure, 6 rats were randomly closed for respiratory function. They were deeply anesthetized by an intraperitoneal injection of 10% Chloral Hydrate (0.3 ml/100 g) before being tracheostomized and tracheocannulated. The cannula was then connected to a 16-channel physiological recorder (Bio-Rad, MP150). Then the lung volume (Tidal Volume TV), frequency (Respiratory Rate) and resistance (Peak Expiratory Flow PEF, Intratracheal Pressure Slope (IP slope) was measured by physiological recorder. All these indexes represented degree of airflow limitation.

After lung function measurement, rats were sacrificed with blooding letting. The right trachea was surgically exposed, and a tracheotomy tube was placed into the right trachea. A silk ligature was fastened around it to secure the tracheotomy tube. Lung lavage was performed to remove bronchoalveolar cells by instilling and withdrawing lavage solution (1.0 mL of 0.9% saline) 3 times via the tracheal tube before finally transferring it to the syringe. Recovery ranged from 70% to 90% of the instilled fluid. There were no differences in the recoveries of the groups. Finally, the lung and liver were removed. The recovered bronchoalveolar lavage (BAL) fluid was centrifuged at 3000 rpm for 10 min, and the supernatant was frozen at −80 °C until further analysis. TNF-a, IL-6, and IL-10 levels in BALF were determined by the ABC enzyme-linked immunosorbent assay system (R&D) according to manufacturer's instructions.

The left lung and the remaining trachea were then fixed for microscopic investigations, tissue specimens with 10% formalin and were stained with haematoxylin and eosin for standard examination. Tissue sections (5–8 μm) were stained and examined under a photomicroscope (Olympus DP71, Tokyo, Japan). All tissue sections were examined by an experienced pathologist (Li Ren, Zhang.) who was blinded to the status of individual animals. Reference to Sener,[18] the histological score of the lung were calculated as the sum of the scores (0–3) given for each criterion, using the semiquantitative scale outlined in Table 1. The maximum score calculated was 9.

Second part

Once the COPD model was established, the remaining rats would be used for second part. This study included a control or a fracture group. Control animals were subjected to 37 weeks persistent tobacco exposure and anaesthesia, whereas fracture animals sustained a unilateral proximal femur fracture using a

Table 1
Criteria for the microscopic scoring of tissue damage.

Tissue	Appearance
Pulmonary	Vascular congestion and interstitial oedema Alveolar structural disturbance Atelectasis and inflammatory cell infiltration
Hepatic	Dilation and vacuolization of hepatocytes Vascular congestion and dilation of sinusoids Kupffer cell infiltration