Research Progress in Modeling Methods of Rat Lung Fibrosis

Xin Zhao\textsuperscript{a}, Zhengmin Cao\textsuperscript{a}, Ruipeng Wang\textsuperscript{b} and Shuang Liu\textsuperscript{c}

Guang’anmen Hospital, China Academy of Chinese Medical Sciences, Beijing
University of Traditional Chinese Medicine, Beijing, China

\textsuperscript{a}Corresponding author e-mail: graycoffee1996@126.com, \textsuperscript{b}751778321@qq.com, \textsuperscript{c}804573973@qq.com

Abstract. Pulmonary fibrosis affects human respiratory function, and severe cases can lead to progressive loss of lung function. Early intervention and early treatment are important for the prognosis of lung fiber. The establishment of pulmonary fibrosis model is of great significance to study the pathogenesis and pathological process of pulmonary fibrosis. Rats are usually selected to model pulmonary fibrosis in various ways. By retrospectively summing up the rat PF model, progress has been made in the study of pulmonary fibrosis.

1. Introduction
Pulmonary fibrosis (PF) is a disease characterized by the proliferation of fibroblasts and the accumulation of a large number of extracellular matrices, accompanied by inflammatory damage and tissue structure destruction, of unknown etiology. It can affect human respiratory function and lead to progressive lung function. Loss, manifested as cough, sputum, wheezing, continuous progress can even develop into respiratory failure, life-threatening. Current research confirms that early intervention and early treatment of pulmonary fibrosis can significantly improve its prognosis. The establishment of pulmonary fibrosis model is of great significance for studying the pathogenesis and pathogenesis of pulmonary fibrosis. In terms of animal selection for animal models of pulmonary fibrosis, rats are the main animal model for PF due to their large size, easy breeding, low cost, and access to a large number of specimens. This article reviews and summarizes rat PF models commonly used in the literature in recent years, and outlines the modeling methods.

By searching keywords such as pulmonary fibrosis, fibrosis model, rat fibrosis modeling method, etc., research and analysis of high-quality literature related to rat lung fibrosis model modeling method in the past three years, establish an excel form to induce Statistics of agents and induction methods were used to form a histogram 1, 2. From the statistical chart, we found that the model of pulmonary fibrosis induced by bleomycin was used up to 296 times, and most researchers used bleomycin Lung fibrosis in rats, the induced model is similar to the individual patient's lung fibrosis, followed by paraquat 55 times and silica 22 times, and the frequency of pulmonary fibrosis induced by silica dust suspension is less. In the induction method, most researchers choose to use tracheal injection. This is the traditional induction method. Its method is simple, and the modeling effect is satisfactory after skilled operation. Some researchers use it by intragastric injection and vascular injection, and only a few of them use it. The researchers chose three methods of nasal instillation, nebulization, and vein to model pulmonary fibrosis in rats.
The separated trachea was fully exposed, and the trachea was injected into the trachea through the cartilage ring. Chloral hydrate was quickly injected into the trachea, and the surgical plate was pulled out. Then, the surgical plate was tied, and the rats were upright and rotated to successfully model the PF model. Immediately afterwards, the rats were upright and rotated to successfully model the PF model.

2. Bleomycin-induced pulmonary fibrosis model
Bleomycin (BLM) is the most widely used PF modeling inducer, which can cause lung damage through direct DNA strand breaks and the generation of free radical-induced oxidative stress. The methods of administration include intratracheal administration, nebulized inhalation, nasal infusion, and tail vein bolus.

2.1. Tracheal administration
Tracheal administration can be performed through surgical incision to expose tracheal instillation and tracheal catheterization. The anesthetics used are slightly different. Common pentobarbital sodium, chloral hydrate, and anhydrous ether are common. Meng Lihong et al. [1] anesthetized the rats with intraperitoneal injection of sodium pentobarbital, fixed their supine position on the surgical board, inserted them into the No. 18 rat catheter through the trachea, and injected bleomycin 3mg/kg. Immediately afterwards, the rats were upright and rotated to successfully model the PF model. Xiong Ting et al. [2] anaesthetized the rats by intraperitoneal injection of chloral hydrate, fixed the rats on the surgical board, and tied the upper incisors of the rats with a string to make their heads lean back. Fully expose the mouth, use oval forceps to pull the rat's tongue to the right, and press the root of the tongue with curved forceps to pull up the mandible. Insert the front hose of the pump into the trachea. After successful intubation, pull out the backward curved forceps and dose A bleomycin solution of 1mg/kg was quickly injected into the trachea, and then 1 to 2mL of air was injected to ensure that the bleomycin completely entered the trachea. Then the catheter was pulled out, and the surgical plate was rotated upright several times. Wang Yuan et al. [3] anesthetized rats by intraperitoneal injection of chloral hydrate, shaved the neck of the rats to disinfect, cut the skin, separated muscle layer by layer, exposed the separated trachea fully, and injected the trachea into the trachea through the cartilage ring space. The erythromycin solution was 5mg/kg, and the PF model was replicated several times by...
quickly standing up and rotating the surgical plate. Hao Meng et al. [4] injected rats with intraperitoneal injection of 10% chloral hydrate for anesthesia, fixed the supine position on the operation panel, exposed and disinfected the rat's neck skin, slightly raised the side of the rat's head, and cut it longitudinally. The skin of the neck was bluntly separated, the trachea was exposed, the tracheal cartilage ring angle was inserted at an angle of 45° with a sterile syringe gap, and 5mg/kg BLM was injected to replicate the pulmonary fibrosis experimental model, rotated upright, and sutured.

Yang Yu et al. [5] made some innovations in the traditional tracheal administration method. During the modeling process, a surgical microscope was used to make a neck incision under the microscope. The incision was about 0.5 cm, and the trachea was exposed bluntly. A bacteria syringe was inserted into the tracheal cartilage space, and 100μl of BLM solution was instilled at 3.5mg/kg. Studies have shown that this modeling method can significantly improve the success rate of modeling, the reliability of the animal model and the work efficiency. Zheng Zhaoxun [6] and others took the lead to use the endoscopic camera monitoring system and the homemade rat anesthesia laryngoscope to bolus bleomycin into the trachea of rats to create a pulmonary fibrosis model. The results showed that this model was used to inject drugs. The target is accurate, the success rate is high, there is no external trauma, the modeling effect is improved, the amount of specimen loss is reduced, and the experimental cost is reduced. Song Jianping [7] used an endoscopic camera monitoring system and a homemade rat anesthesia laryngoscope to anaesthetize the rats with trachea by injecting the drug bleomycin into the lung fibrosis model.

At present, intratracheal administration by surgical incision is the most commonly used method of tracheal administration. The method is relatively simple. After skilled operation, it can evenly distribute the drug solution to each lung lobe of both lungs, and the success rate of modeling is high; The advantages are low, but it is difficult to control the distribution position of the medicinal solution in the lungs, the range of lesions after modeling is limited, and the diffuse distribution of human lesions is different. It will cause local infection and some complications after operation. The success rate of animal models is higher, but the laboratory hardware is higher.

2.2. Nebulization

Atomization can help to evenly distribute the drug solution in the lungs of animals and improve work efficiency, but it requires higher laboratory hardware. Qiu Yue et al. [8] Anesthetized rats with sodium pentobarbital, fixed them on the surgical board, observed the vocal cord opening with a small animal laryngoscope, positioned the trachea, and inserted a hand-held quantitative lung nebulizer via the trachea. The tube was atomized with 150μl of BLM solution at 6mg/kg to successfully establish an animal model of pulmonary fibrosis. Wang et al. [9] used an ultrasonic aerosol device to atomize a 1mg/mL BLM physiological saline solution, and placed the rats in it for 30 minutes to successfully establish a model of pulmonary interstitial epithelial injury and fibrosis in rats.

2.3. Nasal drip

Yang Xingna et al. [10] and Shu Yanmei et al. [11] successfully established a PF model by nasal instillation of bleomycin. Such special infusion methods are mostly used to explore the application of acid suppression therapy and its mechanism of action.

Wang He et al. [12] compared the quality of lung fibrosis models induced by BLM tracheal perfusion, tail vein injection and aerosol inhalation in rats, and randomly divided the rats into normal group, single tracheal perfusion group, multiple tracheal perfusion groups, single intravenous injection group, multiple intravenous injection groups, single nebulization inhalation model group and multiple nebulization inhalation model groups. Observe the effects of the three induction methods and their frequency on the quality of the modeling. Results it shows that the rat lung fibrosis model prepared by inhaling bleomycin multiple times is relatively stable in pathological damage and physiological indicators, which is a relatively efficient method for preparing lung fibrosis model, but different rats inhaled and exhaled at the same time Different, may cause slight differences in the amount of bleomycin inhaled in different animals.
3. **Pingyangmycin-induced pulmonary fibrosis model**

Pingyangmycin-induced pulmonary fibrosis model, the administration method and selection of anesthetic drugs are similar to boramycin.

Study [13] Anesthetized rats with sodium pentobarbital and exposed the skin of the neck after fixation in the supine position. Make a median incision after disinfection, expose the trachea, and use a disposable sterile syringe to inject a 0.4% pingyangmycin physiological saline solution at 5mg/kg in the tracheal cartilage ring space, and immediately sew to prepare a PF model. Liu Lijing [14] also successfully injected pingyangmycin through the trachea to establish a PF model. Liu Yong [15] inserted a disposable trachea through the oropharynx of a rat and injected 0.3 ml pingyangmycin physiological saline solution (5mg/kg) and 0.3ml of air, quickly erected the rotating animal, and successfully made the model. Zhang Shunan et al. [16] used a medical nebulizing ultrasound to inhale nebulized pingyangmycin solution in rats to make a PF model.

4. **Lipopolysaccharide-induced pulmonary fibrosis model**

Lipopolysaccharide (LPS) is the main component of endotoxin. It can cause a series of pathological changes to cause inflammatory response and diffuse interstitial fibrosis in the lung tissue. It can quickly cause pulmonary tissue edema and severe inflammation within 24 to 72 hours. Sexual response, so it is widely used to study the mechanism and treatment of early inflammatory response to acute lung injury induced by endotoxin.

Huang Taibo et al. [17] used a tracheal instillation of 100mg of 3mg/kg lipopolysaccharide in rat trachea to construct a rat model of pulmonary fibrosis in acute respiratory distress syndrome (ARDS); Zhang xiqian et al. [18] and Zhang Juanjuan et al. [19] induced pulmonary fibrosis models by intraperitoneal injection of LPS at 5mg/kg and 10mg/kg, respectively. Xiao Yaqiang et al. [20] used LPS physiological saline solution for nasal drip to replicate the pulmonary fibrosis model after acute lung injury.

4 Silica (SiO2) -induced pulmonary fibrosis model. The damage of SiO2 dust to biofilms mainly composed of alveolar macrophage membrane (AM) is the main cause of silicosis, and the increase of membrane lipid peroxidation caused by SiO2 is considered to be the main cause of membrane damage. Dust cells can release reactive oxygen species (ROS), activate white blood cells to produce ROS free radicals, and undergo a peroxidation reaction with biological membranes, leading to cell membrane damage [21].

Jiang Yan [22] and Zhang Rong [23] used self-made experimental tools to inject 1ml SiO2 suspension through the trachea of the intubated rats. No death during the modeling, histopathological examination and determination of hydroxyproline. The results suggest that the modified tracheal intubation method successfully replicates the pulmonary fibrosis model, which is easy to operate and has low mortality. Liu Yuhong [24], Zheng Jiaoyi [25], Guo Jingwen et al. [26] and other models used PF to inject 0.5ml, 1.0ml, 1.0ml of SiO2 suspension through disposable trachea. Park Xiumei [27], Zhang Enguo et al. [28] used disposable non-exposed intratracheal infusion of sterile silica suspension for modeling. Zhang Chunmei [29] gave rats a 5% sodium pentobarbital solution by intraperitoneal injection of superficial anesthesia, and then trachea exposed perfusion of 0.5ml of 50g/L SiO2 suspension, and PF rats were successfully modeled.

5. **Other animal modeling methods**

Paraquat can cause multiple organ failure, but it is mainly concentrated in the lung. The lung injury is most prominent after poisoning, which can cause early severe pulmonary exudation, delayed pulmonary fibrosis, and pulmonary failure. The mechanism of paraquat-induced lung injury is not clear. At present, many theories that have been studied include oxygen free radical theory, lipid peroxidation damage, cytokine and DNA damage [30].

Wang Kai et al. [31] established a model of pulmonary fibrosis by injecting 0.5ml of a mass concentration of 10 mg mL-1BeO beryllium oxide suspension in a single intratracheal instillation after anesthetizing rats with anhydrous ether. Li Ya et al. [32] used HRH-PM286 PM2.5 real-time online
concentration and enrichment system to keep the rats exposed to PM2.5 for 4 hours per day, and continuously exposed for 14 days and PF rats were successfully modeled. Zhang Honglei [33], Zhang Yin [34], Yue Chongmei et al. [35] used a single intraperitoneal injection of paraquat solution to make a pulmonary fibrosis model to investigate the acute pulmonary fibrosis caused by paraquat.

Song Licheng [36] and other smoke-producing materials are 7 kinds of flammable materials commonly used in indoor fires, including poly shuangma foam materials, foamed rubber and plastic thermal insulation products, flame retardant white glue, damping materials, halogen-free cables, silicon acrylic latex paint, and decorative plates. The smoke generation and experimental device is developed and patented by itself (patent number: ZL 201721317512.6). Grind the smoke material into powder (20g each), place it in a tray above the induction cooker of the smoke box, control the temperature of the induction cooker to 300℃, and after the smoke in the smoke box is filled, use the exhaust fan to pass the smoke through the pipe Drain into the test chamber and use a smoke detector [EM-4L, Australia New Instruments (Hong Kong) Co. Ltd.] to detect the gas concentration in the chamber. The control range of various gas components is: CO (400–550) × 106, H2S (10-15) × 106, O2 18% -20%, the content of the above gas components is maintained by controlling the exhaust fan, and when the gas concentration reaches a preset range, the exhaust fan in the smoke box is turned off; if it does not reach the preset range, turn on the exhaust fan to blow the smoke in the smoke production box into the test box. Except the control group, the smoke inhalation group took 30 minutes as the cause of injury. The smoke in the smoke box was exposed for 30 minutes to meet the criteria for acute lung injury.

In recent years, many advances have been made in the study of animal models of PF. In particular, the pathogenesis of bleomycin is similar to that of clinical PF, and there are fibroblasts and inflammatory changes. It has played an important role in the development of PF drugs and understanding of PF mechanism. However, the clinical course of the disease varies greatly among individual patients with PF in clinical practice, and most patients have an acute episode that causes rapid deterioration of lung function, and pulmonary fibrosis in animal models caused by inducers such as bleomycin. It tends to develop rapidly and at a stable speed, which cannot completely replicate all pathological changes and processes in clinical patients. In addition, the pathology of animal models is different from that of patients with PF. For example, the death of alveolar epithelial cells in PF animal models is a common phenomenon, while the mortality of alveolar epithelial cells in PF patients is low, and the location and severity of inflammation and fibrotic lesions. The degree also varies between patients and animal models. Therefore, no animal model can simulate all PF pathological processes. When studying the pathological process of PF and studying PF preventive and therapeutic drugs, animal models of PF caused by more than one inducer should be selected. We believe that with the deepening of the understanding of PF pathology and the continuous innovation of animal model preparation methods, there will be more animal models in the future that are more in line with the clinical reality of PF.

References
[1]  Meng Lihong, Zhang Xiaomei, Dong Huan, et al.Effect of Yangyin Yiqi Mixture on TGF-β / Smad Signaling Pathway in Pulmonary Fibrosis Rats [J]. Global Chinese Medicine, 2019, 12 (12): 1810-1815.
[2]  Xiong Ting, Xu Xuyan. An experimental study of thalidomide combined with salvia ligustrazure in treating pulmonary fibrosis in rats [J]. Journal of Clinical Pulmonary Medicine, 2019, 24 (12): 2156-2161.
[3]  Wang Yuan, Dai Wenjing, Li Wancheng. Protective effect and mechanism of amifostine on rats with pulmonary fibrosis [J]. Journal of PLA Medical Journal, 2019, 31 (11): 1469-1473.
[4]  Hao Meng, Jia Chunyan, Lu Jun, et al. Therapeutic effects of different prescriptions of Jinqi Decoction on bleomycin-induced pulmonary fibrosis in rats [J]. Journal of Xinjiang Medical University, 2019, 42 (11): 1469-1473.
induced by intratracheal injection of bleomycin [J]. Journal of Xinxiang Medical College, 2015, 32 (09): 807-809.

[6] Zheng Zhaozhang, Liu Enshun, Lian Fu, et al. New exploration of modeling methods of pulmonary fibrosis (qi deficiency and blood stasis syndrome) in rat models [J]. Tianjin Journal of Traditional Chinese Medicine, 2013, 30 (11): 678-680.

[7] Song Jianping, Xie Zhongli, Li Wei, et al. Intervening effects of Guayabai Decoction on hypothalamus, hippocampal DA, NE, 5-HT after rat lung fibrosis (14d-28d) [J]. Chinese Journal of Basic Medicine in Traditional Chinese Medicine, 2011, 17 (06): 624-626.

[8] Qiu Yue, Liu Jinmin, Tang Lei, et al. Effects of Youguiyin on prostaglandins and their receptors in pulmonary fibrosis rats [J]. Journal of Clinical and Experimental Medicine, 2019, 18 (06): 561-565.

[9] Wang Z, Guo QY, Zhang XI. Corilagin attenuates aerosol bleomycin-induced experimental lung injury [J]. Int J Mol Sci, 2014, 15 (6): 9762-9779.

[10] Yang Xingna, Shu Yanmei, Wang Jinliang. Acid suppression therapy in idiopathic pulmonary fibrosis rats with acid aspiration [J]. Chinese Journal of Practical Diagnosis and Therapy, 2019, 33 (04): 330-332.

[11] Shu Yanmei. Experimental study of acid suppression on idiopathic pulmonary fibrosis [D]. Henan University of Science and Technology, 2019.

[12] Wang He, Zhang Guangping, Hou Hongping, et al. Discussion on the model of pulmonary fibrosis in rats induced by different administration methods of bleomycin [J]. Chinese Journal of Experimental Traditional Medical Formulae, 2019, 25 (11): 73-79.

[13] Zhu Feng, Zhai Lihong, Yan Xiaojuan. Effect of antisense matrix metalloproteinase inhibitor-1 gene transfection on oxidative stress-induced pulmonary fibrosis in rats [J]. Journal of Emergency Medicine, 2019, 25 (06): 500-503 + 507.

[14] Liu Lijing, Qian Hong, Hu Ke, et al. MiR-27a-3p inhibits pulmonary fibrosis in rats by blocking Wnt3a / β-catenin pathway [J]. Journal of Cell and Molecular Immunology, 2018, 34 (11): 1015-1020.

[15] Liu Yong, Peng Tangyi, Zhang Xiaoju. Study on the mechanism and mechanism of matrine in inhibiting pulmonary fibrosis in rats [J]. Research and Practice on Chinese Medicines, 2018, 32 (05): 23-26.

[16] Zhang Shunan, Han Chunsheng. A new method of pulmonary fibrosis modeling [J]. Journal of China-Japan Friendship Hospital, 1997, 11 (4): 283-285.

[17] Huang Taibo, Wang Xuelin, Zhang Quncheng, et al. Effect of ambroxol hydrochloride on pulmonary fibrosis in rats with acute respiratory distress syndrome [J]. Chinese Journal of Clinical Pharmacology, 2020, 36 (01): 26-28.

[18] Zhang Qianqian, Wang Yan, An Yunxia, et al. The mechanism of angiotensin II on pulmonary fibrosis in rats [J]. Journal of Xi'an Jiaotong University (Medical Edition), 2019, 40 (03): 352-355 + 361.

[19] Zhang Juanjuan, Hu Shixiang, Li Hua, et al. Regulative effect of ligustrazine on pulmonary fibrosis and inflammatory response in rats with acute lung injury induced by endotoxin [J]. China Hospital Pharmaceutical Journal, 2019, 39 (03): 259-264.

[20] Xiao Yaqiang, Chen Yiping, Xie Zhengfu. Effect of mifepristone on lipopolysaccharide-induced pulmonary fibrosis in rats [J]. Journal of Guangxi Medical University, 2019, 36 (05): 711-715.

[21] Gao Yanxun, Wang Rui. The mechanism of silicon dust-induced fibrosis and the role of cytokines in silicosis fibrosis [J]. Chinese Journal of Industrial Medicine, 2008, 21 (1): 31-35.

[22] Jiang Yan, Zhang Juan, Jia Qiang, et al. Improved method of establishing silicosis rat model by tracheal intubation [J]. Modern Preventive Medicine, 2017 (22).

[23] Zhang Rong, Song Zhanshuai, Zou Jianfang. Effect and mechanism of Buyang Huanwu Decoction on pulmonary fibrosis in silicosis rats [J]. Shandong Medical Journal, 2019, 59 (05): 9-12.
[24] Liu Yuhong, Zhang Shunan, Yuan Feifei, et al. Effects of nourishing qi, nourishing yin, reducing phlegm and activating blood on transforming growth factor-β1 and hydroxyproline content in lung tissue of rats with pulmonary fibrosis [J]. Inner Mongolia Medicine, 2019, 38 (02): 93-94.

[25] Zheng Yijiao, Li Xia, Guo Jingwen, et al. Intervention effect of dihydroartemisinin on pulmonary fibrosis in rats exposed to silicon dust [J]. China Industrial Medical Journal, 2019, 32 (01): 24-27 + 82.

[26] Guo Jingwen. Study on the effect of homemade Chinese medicine and pirfenidone on TGF-β1 / Smad signaling pathway in early stage of silicosis fibrosis in rats [D]. Jinan University, 2019.

[27] Piao Xiumei. Inhibition effect and mechanism of white tea extract on nano-SiO2 induced fibrosis in rats [D]. Zhejiang University, 2019.

[28] Zhang Enguo. Efficacy and mechanism of bone marrow mesenchymal stem cell-derived exosomes in inhibiting silica-induced pulmonary fibrosis [D]. Jinan University, 2019.

[29] Zhang Chunmei, Yang Yongshou, Xiao Peiyun. Effects of active components of Periplaneta americana on lung dust induced by silicon dust in rats [J]. Environment and Occupational Medicine, 2018, 35 (12): 1134-1138.

[30] Sun Jing. Role of TGF-β1 mediated by Hedgehog signaling pathway in paraquat pulmonary fibrosis [D]. Shandong University, 2016.

[31] Wang Kai, Li Sirui, Wang Yuxin, et al. Experimental study of pulmonary fibrosis in rats induced by beryllium oxide [J]. Journal of Ningxia Medical University, 2019, 41 (11): 1091-1095.

[32] Li Ya, Wang Jing, Li Jiansheng, Tian Yange, Liu Xuefang, Feng Suxiang, He Huihui, Jia Rui. Protective effect of pulmonary fibrosis prescription on lung injury induced by PM2.5 in rats [J]. Chinese Journal of Traditional Chinese Medicine, 2019, 34 (06): 2426-2431.

[33] Zhang Honglei. Rosiglitazone inhibits pulmonary fibrosis induced by paraquat in rats and its mechanism [D]. China Medical University, 2019.

[34] Zhang Yin, Liu Yang, He Jingchun, et al. Expression of KL-6 mucoprotein and hydroxyproline and lung fibrosis in lung tissue of paraquat poisoned rats [J]. Journal of Shanxi Medical University, 2019, 50 (02): 149-153.

[35] Yue Chongmei, Wang Songping, Ren Chengji. Intervention effect of losartan on acute lung injury and pulmonary fibrosis in paraquat poisoning rats [J]. Journal of Clinical Pulmonary Medicine, 2018, 23 (12): 2163-2169.

[36] Song Licheng, Han Zhihai, Li Huming, et al. Glucocorticoids reduce pulmonary fibrosis after acute lung injury in rats with smoke inhalation [J]. Journal of Second Military Medical University, 2019, 40 (01): 31-37.