Animal models of emphysema

Gui-Bin Liang, Zhi-Hui He

Department of Intensive Care Unit, The Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, China.

Abstract

Objective: Chronic obstructive pulmonary disease (COPD) is a common chronic respiratory disease of human beings characterized by not fully reversible airflow limitation. Emphysema is the main pathological feature of COPD which causes high mortality worldwide every year and consumes a large amount of medical expenses. This paper was to review the establishment and evaluation methods of animal models of emphysema or COPD, and put forward some new ideas on animal selection, method of modeling, and model evaluation.

Data sources: The author retrieved information from the PubMed database up to July 2019, using various combinations of search terms, including emphysema, model, and animal.

Study selection: Original articles, reviews, and other articles were searched and reviewed for animal models of emphysema.

Results: This review summarized animal models of emphysema from the perspectives of animal selection, emphysema mechanism, modeling method and model evaluation, and found that passive smoking is the classic method for developing animal model of emphysema, mice are more suitable for experimental study on emphysema. Compared with pulmonary function indicators, airway inflammation indicators and oxidative stress indicators, pathomorphological indicators of lung tissue are the most important parameters for evaluating the establishment of the animal model of emphysema.

Conclusions: Mice model induced by passive smoking is the classic animal model of emphysema. Pathomorphological indicators are the most important parameters for evaluating the establishment of the animal model of emphysema.

Keywords: Animal; Chronic obstructive pulmonary disease; Emphysema; Model

Introduction

Chronic obstructive pulmonary disease (COPD) is a common chronic respiratory disease of human beings characterized by not fully reversible airflow limitation.[1] It is mainly caused by cigarette smoke and has a strong impact on human health, seriously affects the quality of patient's daily life, causes high mortality, which brings a heavy economic burden to patients themselves, their families and society. COPD is the fourth leading cause of death worldwide and is expected to rise to third place by 2020.[2] More than 3 million patients died of COPD in 2012, accounting for 6 percent of all deaths worldwide.[3] Buist *et al*[4] found that the prevalence of stage II or higher COPD was 10.1% overall, 8.5% for women, and 11.8% for men. In China, a survey of 20,245 adults in seven regions showed that the prevalence of COPD in people over 40 years old was as high as 8.2%.[5] Globally, the burden of COPD will increase gradually in the coming decades due to the continuing exposure to risk factors and the aging of the population.[6] Although COPD is an important public health problem and the leading cause of chronic disability and death worldwide,[7] it can be prevented and treated.

Significance of Establishing Animal Model of Emphysema

COPD can be induced by many factors, and its mechanisms are very complex including oxidative stress, inflammation, protease-antiprotease imbalance, apoptosis, and even immunosenescence.[8,9] The mechanisms of COPD are not completely illuminated as the exact mechanism by which COPD occurs and progresses remains much of unknown. Regarding ethical issues about the research on COPD patient, COPD animal models is very important for researcher to investigate the mechanism of COPD and the use of COPD animal models is inevitable.[10] Emphysema, as the main pathological feature of COPD, has always been the focus of research which focuses on the mechanism of COPD.[11] Animal models of emphysema improve our understanding of the basic mechanisms of COPD physiology, pathophysiology and treatment.[12] Therefore, to further elucidate the

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000000469

Correspondence to: Dr. Zhi-Hui He, Department of Intensive Care Unit, The Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, China
E-Mail: hzh703@csu.edu.cn

Chinese Medical Journal 2019;132(20)

Received: 13-07-2019 **Edited by:** Xin Chen

etiology and pathogenesis of COPD, many scholars made relevant studies on animal model of emphysema. Although these models only mimic some of the characteristics of the COPD, they are valuable for further study of the mechanisms of human COPD. The establishment and standardization of animal COPD models according to clinicopathology are explored.[13] The establishment of animal models of emphysema is the key to elucidate the etiology and pathogenesis of COPD.[14] In fact, animal models have provided valuable insights into the cellular and molecular mechanisms involved in the pathogenesis of COPD.

Animal Selection for Modeling

Today, a variety of animal models of emphysema have been developed, including sheep,[15] dogs,[16] pigs,[16] rabbits,[17] monkeys,[18] guinea pigs,[19,20] mice[21] rats,[22] and squirrels.[23] Animal models can on some extent reflect the pathology and physiology of human diseases. Most features of COPD, such as inflammatory cell aggregation,[24] oxidative stress,[25] cytokine and protease production,[26] small airway and vascular remodeling,[27] emphysema,[28] pulmonary hypertension,[29] and decreased lung function,[30] can be induced in different models. The development of models of acute exacerbation and complication of COPD can reflect the progress of COPD and provides a powerful tool for the study of the pathogenesis of COPD. However, animals are animals, not human beings. Anatomy, physiology, reactivity to damage, sensibility to cigarette smoke (CS), cytokines, or protease varies from species to species. Lopes *et al*[31] considered that, unlike human progressive COPD, animal exposure to CS only exhibited mild emphysema which did not progress after cessation of exposure.

Anatomy and physiology

Rat model of emphysema was considered to have many advantages, including small body size, low cost of breeding, short reproduction cycle, and similar genome to humans. But rat also has its own limitations. Rats rely on nasal breathing and the nasal cilia have low function on filtering smoke. Furthermore, rats have few branches of the bronchi thus with poor respiratory function. The distribution of intratracheal cilia is less, the glandular submucosal glands are immature, and there are no goblet cells under the tracheal mucosa. The mediators of bronchial inflammation in rats are also different from those in humans.[19]

Pigs have more mature lung tissue than rats, rabbits and other animals, and the volume of lung is relatively large, the lung structure is similar to that of humans with three lobes on the right and two on the left. Even more, pigs, like humans, have respiratory bronchioles, and increased airway resistance of small airways is the main cause of increased airway resistance in patients with COPD. The submucosal glands in the airway of pigs are relatively developed. Compared to other animals, pigs are more prone to respiratory diseases; hence, it is relatively easy to induce COPD. Endotracheal intubation is required for endotracheal infusion drugs, respiratory function evaluation, and simulated assisted ventilation in animals. Compared with rats and guinea pigs, small pigs have larger tracheal diameter, which is convenient for intubation. However, disadvantages of pigs are also obvious, including large body size, high cost of breeding and drug, long reproduction cycle, and less reproduction number. D'Ambrosio *et al*[32] believed that mice were considered to be the best choice for animal model.

Although the distribution of bronchial glands in non-human primates and dogs are roughly similar to that in humans, bronchial glands are limited to the closest part of the trachea. Similarly, primates and dogs have respiratory bronchioles, and their alveolar ducts develop from the membranous bronchioles located at the distal end. The mice have no bronchial circulation.[32] Rodents have much more Clara cells in the small airways than humans.[33] Lung development and maturation are different between different animal species. For example, rats and mice develop their alveoli after birth, while the guinea pig and the monkey are almost fully alveolated at birth. There are differences in the absorption, distribution, metabolism, and elimination of drugs between different species, and these differences may become obstacles in animal modeling.

Reactivity to damage

Most animal models of emphysema induced by passive smoking displayed enlarged alveolar space, although there are various degrees of enlargement from species to species. However, unlike humans, some animals do not develop into serious illnesses, thus narrowing the window by which researcher determines the effects of potential therapeutic interventions.[31] In addition, although goblet cell metaplasia is a characteristic finding of small airways in patients with COPD, the metaplastic response of mice and rats are weaker than those of guinea pigs, dogs and non-human primates.

Sensibility to tobacco

Unlike humans, animal models are an inbred population. Within a species, different strains may respond differently to the same stimulus. In one study,[34] NZWLac/J mice were not sensitive to CS, C57BL/6J and SJ/L mice were somewhat sensitive, and AKR/J mice were more sensitive to CS and exhibited CS-induced COPD. Likewise, DBA/2 mice developed faster emphysema during exposure to smoking, but airway goblet cell metaplasia and mucin 5AC (MUC5AC) expression decreased when compared with C57BL/6J mice.[35]

Cytokines

Common animal models, except monkeys, do not fully replicate human cytokines, which are magnified when compared to rodents. For example, in rodents, the closest cytokines match to human interleukin (IL)-8 are chemokines KC (CKC) and cytokine-induced neutrophil chemotactic factors (CINCs), and CINC has better homology to human melanoma growth stimulatory activity (MGSA)/gro. Different strains within the same species may have different inflammatory cell and cytokine response profiles for the same stimulus.[36]

Proteases

Matrix metalloproteinases (MMP)-12 is a major component of mice macrophage metalloproteinases, but for humans, MMP-7 appears to be more important in destroying elastic tissues.[37]

Among the animals described above, although mice emphysema model could not be the exact replica of human emphysema because of differences between human and mice in both physiological, immune and anatomical systems.[38] Mice genome is more similar to that of human than many other animals,[39] and offers the advantages of extensive gene/protein sequence/antibody availability, low cost, and most importantly, the availability of many naturally occurring mouse strains for different responses to smoking. Hence, mice are considered to be more suitable for experimental study on emphysema.

Mechanisms of Emphysema

Elastase-antielastase imbalance

The destruction of large amounts of proteins in the pathogenesis of COPD attracted researchers' attention since 1970s. There is an imbalance between elastases (MMP family) and anti-elastases, which break down proteins in lung tissue of COPD patients. Too much of elastases released by patient's inflammatory cells can cause damage to lung parenchyma, resulting in emphysema. Direct evidence is that the lack of genetic alpha 1-antitrypsin (AT) causes the elastase-antielastase imbalance that produces emphysema-like changes.[40] Under the repeated attack of chronic inflammation, lung tissue is constantly damaged and repaired. Due to the persistence of inflammatory and other damage factors, abnormal growth occurs in the process of tissue repair, such as remodeling of connective tissue fibers in alveoli. The hardness and biomechanical characteristics of remodeled tissue fibers are significantly lower than those of normal tissues, and the lung elasticity is decreased, which may be one of the reasons for the further development of emphysema.

Oxidation-antioxidant imbalance

Oxidation-antioxidant imbalance is an important mechanism for the occurrence of COPD, and oxidative stress further increases with acute exacerbation of COPD. CS and other harmful particles can produce excessive oxides when being inhaled into body, which can directly damage lung tissue.[41] Oxides mainly consist of superoxide anion (O^{2-}), hydroxyradical (OH), hypochlorous acid (HClO), H_2O_2 and nitric oxide (NO). Oxide can not only directly destroy a lot of biochemical macromolecules such as protein, lipids, and nucleic acid to make cell dysfunctional and even dead, but also destroy extracellular matrix to cause protease-antiprotease imbalance.[42] Some antioxidative expectorants, such as endostatin, carboxymethyl steam, n-acetylcysteine, and ambroxol can alleviate the acute exacerbation of COPD, slow down the decline of pulmonary function,[43] which indicate that antioxidant may play a role in the onset and progression of COPD. In addition, these antioxidants have been applied to animal studies to reduce emphysema.[44] At present, the issue how to develop more effective antioxidant becomes one of the research priorities for COPD prevention.

Inflammatory mechanism

Inflammation has been recognized as the most important mechanism both at the beginning of COPD and the progress of COPD. Under normal circumstances, the human respiratory tract, including the nose, pharynx, larynx, trachea, and bronchi, has appropriate defensive function. The structures that perform the defensive function include the nasal hair in nasal cavity, normal cough reflex in the throat, the cilia on the surface of the tracheo-bronchi and the mucus removal system composed of mucus. These structures are looked as the natural barriers which defend human body against foreign invader. The normal reflex of guttur ministry can prevent foreign material entering airway instinctively and keep the lower airway bioclean. If these defense mechanisms fail to work, foreign particles (including dust particles, bacteria and other microorganisms) will enter the lower respiratory tract, activate macrophages, neutrophils and lymphocytes in lung tissue, release a variety of media, including leukotriene B4 (LTB4), IL-8, tumor necrosis factor (TNF)-α, intercellular adhesion molecule (ICAM) 1 and transforming growth factor (TGF)-β, etc.[45] These mediators can damage lung tissue, promote neutrophil inflammation, which activates neutrophil cells to migrate to inflammatory sites, causing ciliated bronchial epithelial cells to become mucous goblet cells, damaging lung septum, and enlarging the alveolar cavity.

Hormone related mechanism

Inflammatory mechanism exists in COPD patients. Except for the acute exacerbation stage, corticosteroid therapy in COPD patients is rarely effective, that is called "hormone resistance," and the mechanism is unclear. Birrell *et al* found that the hormonal resistance in COPD may be related to the inactivation of κB pathway.[46] Other study[47] found that the activity of histone deacetylase in the lung of COPD patients was decreased and negatively correlated with the severity of the disease, suggesting that the respiratory hormone resistance of patients may be related to the decreased activity of histone deacetylase.

Immunologic mechanism

Macrophages are the major phagocytes and they can engulf foreign particles and pathogens, release cytokines which can not only enhance the phagocytosis of macrophages, but also do some harm to human body.[47] Lymphocytes are also involved in the pathogenesis of COPD. $CD8^+$ lymphocyte family members play a very important role in the pathogenesis of COPD.[48] Even after smoking cessation, the inflammatory response in the lungs does not stop, but continues to progress.

Vagus nerve stimulation

Vagus nerve excitation is present in the pathogenesis of COPD. COPD patients have the characteristics of high

airway reactivity, abnormal increase of cholinergic nerve tension and enhanced cholinergic nerve reflex. The reasons may lie in that the nerve transmission in the cholinergic ganglion is enhanced, which makes the release of acetylcholine increased. At the same time, the airway is more responsive to endogenous acetylcholine, and the dysfunction of inhibitory feedback regulation caused by the low function of muscarinic (M) receptor is another reason for hypercholinergic function.[49] The increased vagus nerve tension leads to the contraction of bronchial smooth muscle, which is mainly due to the increased expression of various signal molecules in the M-receptor-mediated airway smooth muscle and the excessive release of neuroacetylcholine caused by inflammation related neurogenic mechanism. Meanwhile, the increased vagus nerve tension causes hypersecretion of glands under airway mucosa. Acetylcholine could come from parasympathetic nervous system, bronchial epithelial cells, inflammatory cells and other cells,[50] and a variety of inflammatory cells express functional M receptors, participating in the regulation of airway inflammation. In addition, acetylcholine can induce the proliferation of fibroblasts and myofibroblasts, playing a prominent role on airway remodeling. Therefore, the cholinergic mechanism is of great significance in the pathophysiology of COPD.

Modeling Methods of Animal Model of Emphysema

Elastase induced animal model of emphysema

Emphysema could be induced by one or more drops of elastase into the trachea.[51,52] This method is relatively simple to operate and can shorten the experiment period and save the cost. The instillation of elastase disrupts protease-antiprotease balance in lung tissue, which not only destroys the main factors that protect lung tissue from damage, but also produces a large number of inflammatory factors and accelerates the rupture and fusion of alveolar walls to induce emphysema.[53] Commonly used elasteases are: papain,[54] pig pancreatic elastinase (PPE),[55] and human neutrophil elastase (HNE).[56]

Papain is a proteolytic enzyme from plants and the earliest elastase used to induce emphysema model.[57] In 1960s, papain was used to successfully set up an rat model of emphysema for the first time.[58] In 1980s, Boyd *et al*[59] discussed the dosage of papain used in rat model and set the dosage at 2 or 4 mg/kg, but the results showed that there was no significant difference in emphysema-like lesions between the two different dose groups. So it was considered that one-time infusion of papain with 2 mg/kg into the trachea was a relatively reasonable dose.[59] Sulkowski *et al*[60] also induced stable emphysema model by one-time infusion of 2 mg/kg papain into the trachea of rats. Thereafter, papain was used to induce rat model of emphysema at a dose of 2 mg/kg. Interestingly, emphysema could also be induced by exposure to aerosol of 10% papain for 8 h twice in a 2-week interval.[61]

The commonly used PPE for animal model of emphysema was derived from swine pancreas.[55] PPE can not only act as a protease to destroy protease-antiprotease imbalance, but also act as an oxidant to induce oxidative stress. With the double effect described above on the progress of emphysema, the alveoli in the experimental animal model were significantly enlarged. Therefore, PPE was often used to induce emphysema. PPE concentrations range from 6 to 24 U. The usage of PPE induction mainly includes intratracheal drip, tracheotomy injection, atomization inhalation. Generally, it usually takes about 4 to 6 weeks to induce emphysema-like changes.[60]

HNE is serine protease, which plays a major role in the COPD inflammatory process. The protease/anti-protease imbalance leads to an excess of extracellular HNE hydrolyzing elastin and structural protein that confers elasticity to the lung tissue.[62] Due to the weak ability of HNE[20] to enter the alveolar septum and degrade elastic fibers, HNE is seldom used at present to induce emphysema.

Raub *et al*[63] showed that the hamster with intratracheal injection of 6, 12, or 24 units of PPE exhibited a dose-related change in lung function after 4 weeks, suggesting that in hamsters, six units of elastase could produce mild emphysema. The method of using protease to replicate animal model of emphysema has the advantages of less infection, easy to grasp the dosage and short period. Therefore, direct intratracheal administration of protease is an effective way to induce animal model of emphysema.

Passive smoking induced animal model of emphysema

According to clinical statistics, about 90% of COPD patients were smokers.[64] One of the most important risk factors for emphysema is smoking.[65] In 1990, Wright *et al*[66] successfully set up guinea pig model of emphysema by means of CS exposure for the first time. He found that the long-term smoking will lead to the changes in the center of the human lobules, causing emphysema. Animal with long-term CS exposure could result in inflammatory response in lungs, which was mainly composed of macrophages.[67] As a result, the bronchial lumen narrowed and the bronchial cartilage tissue was impaired, leading to the rupture and fusion of alveoli and the formation of emphysema, which was similar to human beings' response to smoking. Passive smoking induced emphysema can simulate the pathogenesis of human emphysema as much as possible, and provide a foundation for the basic and clinical research of human emphysema. The structure of the airway and lung of experimental animals are different from species to species, and from that of human beings. The guinea pig is the most sensitive animal to the smoke stimulation, and the rats show a certain resistance to the smoke stimulation, but the susceptibility to smoke is also different in different species of rats. The experimental period of passive smoking induced COPD animal model is relatively long and the stability is relatively poor.[68]

According to the literature review, the exposure to CS can be roughly divided into two types: one is the part exposure (nose or head only) method.[69] Van der Strate *et al*[70] studied on C57BL/6J mice which inhaled CS through their noses for 2 times/day, 2 cigarettes/time, 10 spray/cigarette, 5 days/week. The results showed that after 4 months of exposure, the pulmonary alveoli enlarged with the increase

of smoking time. At the same time, B lymphocyte in lung tissue of the smoked mice increased similar to what was seen in human emphysema. The other exposure method is the whole body exposure method,[71] in which the experimental animal is placed in a smoking box (a box full of smoke) as a whole. Valenca *et al*[72] exposed C57BL/6 mice to cigarette smoke for 3 cigarettes/time and 3 times/day. After 60 days, emphysema-like changes in lung were observed, which were accompanied by increased alveolar macrophages, extracellular matrix changes and increased expression of MMP-12. Our previous study established mice model of emphysema by exposing C57BL/6J mice to CS in a smoking box with some hole on it. In the box, a partition with the same size holes was placed in the middle of the box to divided it into two parts: the lower part was used for cigarette burning, and the upper part was used for animal exposure to CS. Mice were exposed for 2 cycles/day, 6 days/week for 12 weeks.[73] The passive smoking method is quite popular due to its low cost, simple operation, high success and can eliminate the experimental differences in a more objective environment[74]

The length of CS exposure for animal model of emphysema might be due to the different kind of cigarette, different exposure mode, duration and frequency, different smoke density, different species and age of animals and so on.

Chemicals induced animal model of emphysema

Many chemicals, including NO_2, lipopolysaccharides (LPS),[75] O_3, and cadmium chloride ($CdCl_2$), intravenous injection of hyaluronidase,[76] inhalation of ovalbumin dry powder,[77] could cause inflammation and emphysema. NO_2, which is common in air pollution, can induce the animal model of emphysema by controlling the concentration and inhalation time of NO_2. Wegman *et al* found that animal emphysema models could also be set up by oxidative stress after long-term exposure of mice to NO_2 with a volume fraction of 20×10^6, which lasts 14 h a day for 25 days.[78] LPS caused airway and lung tissue inflammation mainly through stimulating neutrophils, monocytes and endothelial cells which released a series of inflammatory mediums including TNF-α, IL-1, etc, triggering protease-antiprotease imbalance, eventually emphysema occurred.[79] Snider *et al* found that animal model of emphysema could be induced by one-time dropping 0.5 mL 0.025% $CdCl_2$ solution into the trachea of golden ground squirrels.[23]

Cigarette smoke extract induced animal model of emphysema

In 2006, Taraseviciene-Stewart and coworkers[80] reported that intraperitoneal injection of cigarette smoke extract (CSE) produced significant emphysema in mice. They hypothesized that CSE could act as an antigen to trigger an immune response, leading to emphysema. It took only 6 weeks to establish a model of emphysema. The problem whether CSE impairs lung tissue targeted making inflammatory cells homing in focus or the systemic inflammatory cells induced by CSE infiltrate in the lung tissue through the impaired endothelium is unclear and needs further study. Even more, in 2009, Chen *et al*[81] reported that intraperitoneal injection of CSE in rats could induced emphysema-like injury within 3 weeks. Although it has been confirmed in these reports that intraperitoneal exposure to CSE was able to cause emphysema in animals, extrapulmonary effects were underestimated. Our research team established mice model of emphysema by intraperitoneal injection of CSE and fully evaluated the model.[73,82-85] The total experimental period was four weeks. On day 29, the mice were disposed for lung function measurement, blood collection, bronchoalvolar lavage (BAL) and histomorphological detection of lung tissue. The results demonstrated that intraperitoneal injection of CSE could lead to pulmonary function decline, alveolar space increase, alveolar wall destruction, apoptosis of alveolar septum cells, chronic lung inflammation, decreased serum superoxide dismutase (SOD) concentration, and elevated IL-6 concentration in animal model. More importantly, the effectiveness of this modeling methods was equal to that of CS exposure.[73] Our previous study described the preparation of CSE in details including the content of nicotine and carbon monoxide in the cigarette.[86]

Other exogenous factors induced animal model of emphysema

It has also been reported that emphysema-like changes could be detected in the case of accelerated metabolism of elastic fibers and collagen fibers in lung tissues due to severe hunger. Sahebjami *et al*[61] found that taking less food (for a third of control group) could induced emphysema, the number of alveoli, lung volume, alveolar lining area changed significantly, and the animal's body weight decreased to 40% to 45% of normal, but there was no increased number of neutrophils in lung tissue. The authors believed that it may be because of long-term starvation that the growth and development of lung tissues in experimental animals were disturbed, which could not reflect the real destruction process of lung tissues in human emphysema. So this method is rarely used.

Genetic manipulation in animal model of emphysema

In recent years, with the development of human genome project and molecular biology technology, the relationship between diseases and genes has been deeply studied. Many scholars believed that the corresponding animal models of emphysema could be induced by regulating the emphysema-related genes which activates some new explorations in the research field.

Natural variation in animal model of emphysema

Spontaneous emphysema was first discovered in spotted mice in 1970s, and it was believed that spontaneous emphysema was mainly related to the abnormal mechanism of connective tissue, collagen and elastin cross-linking. In long-term animal experiments, it was found that the spontaneous emphysema of mice may be Tit-skin mice, Beige mice, Blotchy mice, Palliad mice, etc.[87]

Gene-knockout in animal model of emphysema

With the development of molecular biology, the animal model of emphysema induced by gene knockout has been

widely used in the research of emphysema. In recent years, an increasing number of studies used gene knockout to copy animal model.[88-90] Baron *et al* outlined the major technological approaches to the utilization of gene-targeted and/or genetically modified mice to delineate the cellular and molecular basis of experimental lung disease.[91] Liang *et al*[92] found Abhd2 knockout mice exhibited emphysema-like changes in lung due to the excessive inflammatory cytokines and protease gene expression, increased macrophages, abnormal apoptosis, and resistance to the lack or loss of protease inhibitors. And the copies showed a gradual progress of emphysema in a similar way in occurrence, development process and clinical pathology. Therefore, it is of great significance to study the genetic susceptibility and environmental factors of emphysema.[93]

Other gene-associated animal model of emphysema

Platelet derived growth factor-β (PDGF-β), TNF-α, IL-6 and IL-11 could interfere the normal development of alveoli.[94] Previous study found that if the expression of some corresponding genes were extremely increased in the process of growth and development of mice, alveolar developmental disorders will happen, leading to the formation of emphysema because excessive expression of certain genes may disrupt the balance between alveolar damage and repair, leading to emphysema.[95] TNF-α is an immunomodulatory factor secreted by monocytes and macrophages, which could induce inflammatory cells.[96] It also plays a role in the synthesis of IL-6, IL-8, prostaglandin, leukotriene and other secondary inflammatory mediators. Appropriate expression of gene is necessary to maintain the homeostasis in human body, excessive expression will aggravate the inflammatory response.[94] In 1999, Hoyle *et al*[97] developed transgenic mice with the PDGF-β gene. The transgenic mice displayed many pathological changes in lung including dilation of alveolar cavity, rupture of alveolar wall, fusion of alveoli, inflammatory reaction. It was suggested that the replication of emphysema models in experimental animals could be achieved by overexpression of PDGF-β. Study on the lung tissue derived from homozygous mutant Klotho mice[95] showed that mice with Klotho gene disruption had enlarged distal alveolar cavity at 4 weeks of age accompanied by damaged alveolar wall and progressive aggravation with age, which was very similar to senile emphysema. Based on the study of emphysema-related gene, MMP-1 was found to be activated in the lungs of emphysema patients, and it was considered that transgenic mice with MMP-1 could also display emphysema-like lesions.[98] Many scholars believed that MMP-1, secreted by alveolar type II epithelial cells, may be the leading cause of continuous destruction of lung tissue.[98,99]

The advantages and disadvantages of animal models induced by the modeling methods described above were summarized in Table 1. Since the emphysema itself is a chronic progressive disease, short period of modeling is an advantage as well as a disadvantage.

Table 1: Advantages and disadvantages of each animal model.

Models	Advantages	Disadvantages
Elastase induced animal model of emphysema	Simple operation, short period of modeling and low costs.	1. Not consistent with the process of human emphysema. 2. The acute effects of elastase instillation are different from the chronic progress of human emphysema.
Passive smoking induced animal model of emphysema	Similar to smoking. Animal's airflow obstruction and decreased compliance of respiratory system occur and progress slowly. Low costs.	The period of modeling is relatively long.
Chemicals induced animal model of emphysema	Simple operation, short period of modeling and low costs.	1. Not consistent with the process of human emphysema. 2. The short period of modeling is different from the chronic progress of human emphysema.
Cigarette smoke extract induced animal model of emphysema	Simple operation, short period of modeling and low costs.	1. Not consistent with the process of human emphysema. 2. The short period of modeling is different from the chronic progress of human emphysema.
Other exogenous factors induced animal model of emphysema	Simple operation, short period of modeling and low costs.	1. Not consistent with the process of human emphysema. 2. The long-term starvation induced emphysema cannot reflect the exact mechanisms of human emphysema.
Genetic manipulation in animal model of emphysema	Able to clarify the influence of various genes on emphysema.	1. High requirement on technology. 2. High cost.

Animal Model of Acute Exacerbation of COPD

An acute exacerbation of COPD (AECOPD) is defined as acute worsening of respiratory symptoms and requiring additional treatment.[100] AECOPD can directly lower the quality of patient's daily life, lead to high mortality. So, animal model of COPD Exacerbation is of great value in investigating the pathogenesis of AECOPD.

AECOPD animal models can be roughly divided into three types, including LPS,[101] bacterial,[102] and virus.[103] A single large dose of LPS can cause an inflammatory response accompanied by fever, excessive mucus secretion, and bronchoconstriction, resulting in an AECOPD animal model. Animal model of LPS-induced exacerbation has been established in hamsters. Basic emphysema was established through elastase administration and subsequently LPS was applied twice a week for 5 weeks to evoke exacerbation. After 6 months, the AECOPD animal model exhibited severe mucus cell hyperplasia and serious alveolar enlargement which measured by mean linear intercept (MLI) and bronchial mucus cell hyperplasia (BMCH), scored in tissue slice stained with periodic acid-Schiff.[104]

More than half of the acute exacerbations of COPD are caused by bacterial infections. The more severe the patient is the more species of bacteria could be derived from the patient. Few study used bacteria induced animal model of AECOPD to study human's AECOPD, although the animal model may display obviously increased inflammatory responses. Compared with mice exposed to normal air, mice infected with Haemophilus influenzae after 8 weeks of exposure to CS had an increased inflammatory response and worsened lung injury.[102] Huvanne *et al*[105] studied on the animal model which were exposured to CS for 4 weeks and with nasal administration of staphylococcus aureus enterotoxin B (SEB) for the next 2 weeks. The results demonstrated that the animal exposure to both CS and SEB exhibited increased inflammatory cells in the lung when compared with the animal exposure to either CS or SEB alone.

Patients with COPD have an increased susceptibility to influenza A virus (IAV) infection and an enhanced inflammatory immune response to infection. In the acute or chronic CS exposure induced animal models, an increased local and systemic inflammation were observed, which were followed by IAV infection. In some patients, viral proliferation increases or clearance decreases, and bronchodilator response decreases. Donovan *et al*[103] placed mice in an 18 L perspex chamber and exposed them to CS which was generated from nine Winfield Red cigarettes (<16 mg tar, <1.2 mg nicotine and <15 mg of carbon monoxide) per day for 4 days. On day 5, mice were anaesthetized by inhalation of methoxyflurane and infected intranasally with 10 plaque forming units (PFU) of the mildly virulent influenza A virus Mem 71 (H3N1). On day 12, it was demonstrated that virus inducd animal model of AECOPD was established.

Evaluation on Animal Model of Emphysema

After the establishment of the animal model of emphysema, corresponding evaluation methods are required to confirm the success of the model. There was global strategy for the diagnosis and classification of COPD in human,[3] but no for animal emphysema or COPD. The commonly used parameters include pulmonary function indicators, airway inflammation indicators, oxidative stress indicators and pathomorphological indicators. Lung function indicators include recording airway resistance (Raw), lung dynamic compliance (Cdyn), peak expiratory flow (PEF), inspiratory time/expiratory time (Ti/Te),[106-110] and blood gas analysis.[111] Airway inflammation indicators include cell count and classification in alveolar lavage fluid, neutrophils, macrophages, eosinophils,[112-114] TGF-β,[115,116] IL-6,[117] IL-8,[118] TNF-α,[117] leukotriene B4 (LTB4),[119] elastolytic enzymes such as MMP-1, MMP-2, MMP-9, MMP-12,[120] and cathepsins K, L, S,[121] and monocyte chemotactic protein 1 (MCP-1).[122] Oxidative stress indicators include SOD,[118] reactive oxygen species (ROS),[123] and nuclear factor correlation factor 2.[124] Pathomorphological indicators include mean linear intercept (MLI), destructive index (DI), apoptotic index (AI),[73,84,117,125,126] and pathologic score of airway.[127] According to the American Thoracic Society, emphysema was defined as "abnormal, permanent enlargement of the airspaces distal to the terminal bronchiole, accompanied by destruction of their walls".[128] Donaldson *et al*[129] considered the most important pathological and physiological changes of COPD are the obstruction of airway and the decline of lung function.[129] An ideal animal model of COPD should be in line with clinical practice, such as the injury factors consisted with the common causes of clinical COPD, airflow obstruction, decreased lung dynamic compliance, airway remodeling, and airway hyperreactivity. However, pulmonary function tests were considered to be less sensitive than morphometry and might detect only more severe degrees of airways remodeling or parenchymal destruction. Mild emphysema animal model might have normal lung function.[130] Ochs suggested that the quantitative assessment of micro-structure was the only way to reliably demonstrate the presence of emphysematous alterations.[131] So far, there is no uniform standard in the evaluation system. In consideration of the accessibility, objectivity, and stability of various parameter, we believe that the changes in pathomorphological indicators, including MLI, DI, AI, are the most important parameter for evaluating the establishment of the animal model of emphysema.

Summary and Prospect

Various animal models of emphysema have been developed, but there is no animal model which can simulate all the characteristics of human COPD. According to the mechanism of COPD, the evaluation method for emphysema animal model is based on pulmonary function, pathomorphism of lung tissue, airway inflammation.

Although several emphysema animal models have been established, exact comparisons of findings from various groups are difficult because different methods, different chemicals, different types of chemicals or cigarettes, different doses of cigarette smoke, different instruments, different exposure protocols, and a wide variety of animals are used. Cigarette smoking is by far the most important

risk factor for emphysema and COPD. CS exposure was regarded as the traditional method of long-term modeling of emphysema. CS exposure induced emphysema can simulate relatively complex pathological changes and is considered as the most reasonable animal model of COPD at present. Because of the long modeling time, inconsistence and unstability, researchers have constantly explored new modeling methods.

Up to now, there is no perfect experimental animal model of emphysema which is completely consistent with the pathogenesis and characteristics of human emphysema. Although mice and humans share many basic physiological processes, specific differences in lung structure, function and immunology between humans and mice have to be taken into consideration. Even within mice, different strains exhibit different sensitivities to the development of emphysema. With the continuous in-depth study of emphysema, there are more and more alternative induction methods. Therefore, we should not only induce corresponding experimental animal models of emphysema according to the requirements of experimental purposes, but also explore the pathogenesis of emphysema through multiple methods of modeling. We believe that with the development of science and technology, more reasonable and standardized animal models of emphysema will be applied to experimental research in the near future.

Funding

This study was supported by a grant from the National Natural Science Foundation of China (No. 81870040).

Conflicts of Interest

None.

References

1. Alfageme I, Reyes N, Merino M, Reina A, Gallego J, Lima J, *et al*. The effect of airflow limitation on the cause of death in patients with COPD. Chron Respir Dis 2010;7:135–145. doi: 10.1177/1479972310368692.
2. Cavaillès A, Brinchaultrabin G, Dixmier A, Goupil F, Gutgobert C, Marchandadam S, *et al*. Comorbidities of COPD. Eur Respir Rev 2013;22:454–475. doi: 10.1183/09059180.00008612.
3. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, *et al*. Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease 2017 Report. GOLD Executive Summary. Am J Respir Crit Care Med 2017;195:557–582. doi: 10.1164/rccm.201701-0218PP.
4. Buist AS, Mcburnie MA, Vollmer WM, Gillespie S, Burney P, Mannino DM, *et al*. International variation in the prevalence of COPD (The BOLD Study): a population-based prevalence study. Lancet 2007;370:741–750. doi: 10.1016/s0140-6736(07)61377-4.
5. Yang G, Wang Y, Zeng Y, Gao GF, Liang X, Zhou M, *et al*. Rapid health transition in China, 1990-2010: findings from the Global Burden of Disease Study 2010. Lancet 2013;381:1987–2015. doi: 10.1016/S0140-6736(13)61097-1.
6. Patrick Mallia MD, Johnston SL. COPD: new treatment strategies. Future Prescriber 2013;14:13–16. doi: 10.1002/fps.104.
7. Arne M, Lundin F, Boman G, Janson C, Janson S, Emtner M. Factors associated with good self-rated health and quality of life in subjects with self-reported COPD. Int J Chron Obstruct Pulmon Dis 2011;6:511–519. doi: 10.2147/COPD.S24230.
8. Cho WK, Lee CG, Kim LK. COPD as a disease of immunosenescence. Yonsei Med J 2019;60:407–413. doi: 10.3349/ymj.2019.60.5.407.
9. Dahl M, Nordestgaard BG. Markers of early disease and prognosis in COPD. Int J Chron Obstruct Pulmon Dis 2009;4:157–167. doi: 10.2147/copd.s3106.
10. Andersen ML, Winter LMF, Andersen ML, Winter LMF. Animal models in biological and biomedical research - experimental and ethical concerns. An Acad Bras Cienc 2019;91:e20170238. doi: 10.1590/0001-3765201720170238.
11. Mamoru T, Gen Y, Hiroyuki K, Hiroki T. Classification of centrilobular emphysema based on CT-pathologic correlations. Open Respir Med J 2012;6:155–159. doi: 10.2174/1874306401206010155.
12. Ghorani V, Boskabady MH, Khazdair MR, Kianmeher M. Experimental animal models for COPD: a methodological review. Tob Induc Dis 2017;15:25. doi: 10.1186/s12971-017-0130-2.
13. Jones B, Donovan C, Liu G, Gomez HM, Chimankar V, Harrison CL, *et al*. Animal models of COPD: what do they tell us? Respirology 2017;22:21–32. doi: 10.1111/resp.12908.
14. Masaki F, Yoichi N. The pathogenesis of COPD: lessons learned from in vivo animal models. Med Sci Monit 2007;13:19–24. doi: 10.1051/medsci/2007232221.
15. Abraham WM. Modeling of asthma, COPD and cystic fibrosis in sheep. Pulm Pharmacol Ther 2008;21:743–754. doi: 10.1016/j.pupt.2008.01.010.
16. Chapman RW. Canine models of asthma and COPD. Pulm Pharmacol Ther 2008;21:731–742. doi: 10.1016/j.pupt.2008.01.003.
17. Mata JF, Altes TA, Cai J, Ruppert K, Mitzner W, Hagspiel KD, *et al*. Evaluation of emphysema severity and progression in a rabbit model: comparison of hyperpolarized 3He and 129Xe diffusion MRI with lung morphometry. J Appl physiol 19852007;102:1273–1280. doi: 10.1152/japplphysiol.00418.2006.
18. Plopper CG, Hyde DM. The non-human primate as a model for studying COPD and asthma. Pulm Pharmacol Ther 2008;21:755–766. doi: 10.1016/j.pupt.2008.01.008.
19. Wright JL, Churg A. Animal models of cigarette smoke-induced COPD. Chest 2002;122:301s–306s. doi: 10.1378/chest.122.6_-suppl.301s.
20. Mahadeva R, Shapiro SD. Chronic obstructive pulmonary disease * 3: Experimental animal models of pulmonary emphysema. Thorax 2002;57:908–914. doi: 10.1136/thorax.57.10.908.
21. Brusselle GG, Bracke KR, Maes T, D'Hulst AI, Moerloose KB, Joos GF, *et al*. Murine models of COPD. Pulm Pharmacol Ther 2006;19:155–165. doi: 10.1016/j.pupt.2005.06.001.
22. Martin JG, Tamaoka M. Rat models of asthma and chronic obstructive lung disease. Pulm Pharmacol Ther 2006;19:377–385. doi: 10.1016/j.pupt.2005.10.005.
23. Snider GL, Lucey EC, Faris B, Junglegg Y, Stone PJ, Franzblau C. Cadmium-Chloride-induced air-space enlargement with interstitial pulmonary fibrosis is not associated with destruction of lung elastin: implications for the pathogenesis of human emphysema. Am Rev Respir Dis 1988;137:918–923. doi: 10.1164/ajrccm/137.4.918.
24. Kai MB, Beier J, Kornmann O, Mander A, Buhl R. Long-term repeatability of induced sputum cells and inflammatory markers in stable, moderately severe COPD. Chest 2003;123:778–783. doi: 10.1378/chest.123.3.778.
25. Rahman I, Morrison D, Donaldson K, Macnee W. Systemic oxidative stress in asthma, COPD, and smokers. Am J Respir Crit Care Med 1996;154:1055–1060. doi: 10.1164/ajrccm.154.4.8887607.
26. Soodaeva S, Klimanov I, Li T, Boldina M, Postnikova L, Kubysheva N. Production of proinflammation cytokines and mean pulmonary arterial pressure in COPD. 2013. Available from: https://erj.ersjournals.com/content/42/Suppl_57/P643.article-info. [Accessed July 25, 2019].
27. Hashimoto M, Tanaka H, Abe S. Quantitative analysis of bronchial wall vascularity in the medium and small airways of patients with asthma and COPD. Chest 2005;127:965–972. doi: 10.1378/chest.127.3.965.
28. Gelb AF, Hogg JC, MüLler NL, Schein MJ, Kuei J, Tashkin DP, *et al*. Contribution of Emphysema and Small Airways in COPD. Chest 1996;109:353–359. doi: 10.1378/chest.109.2.353.
29. Minai OA, Chaouat A, Adnot S. Pulmonary hypertension in COPD: epidemiology, significance, and management: pulmonary

vascular disease: the global perspective. Chest 2010;137:39S–51S. doi: 10.1378/chest.10-0087.
30. Li N, Ouyang BS, Liu L, Lin CS, Xing DD, Liu J. Dexmedetomidine protected COPD-induced lung injury by regulating miRNA-146a. Bratisl Lek Listy 2016;117:539–542. doi: 10.4149/BLL_2016_106.
31. Lopes FD, Toledo AC, Olivo CR, Prado CM, Leick EA, Medeiros MC, *et al*. A comparative study of extracellular matrix remodeling in two murine models of emphysema. Histol Histopathol 2013;28:269–276. doi: 10.14670/HH-28.269.
32. D'Ambrosio D, Mariani M, Panina-Bordignon P, Sinigaglia F. Chemokines and their receptors guiding T lymphocyte recruitment in lung inflammation. Am J Respir Crit Care Med 2001;164:1266–1275. doi: 10.1164/ajrccm.164.7.2103011.
33. Fahy JV, Corry DB, Boushey HA. Airway inflammation and remodeling in asthma. Curr Opin Pulm Med 2001;6:15–20. doi: 10.1097/00063198-200001000-00004.
34. Guerassimov A, Hoshino Y, Takubo Y, Turcotte A, Yamamoto M, Ghezzo H, *et al*. The development of emphysema in cigarette smoke-exposed mice is strain dependent. Am J Respir Crit Care Med 2004;170:974–980. doi: 10.1164/rccm.200309-1270OC.
35. Bartalesi B, Cavarra E, Fineschi S, Lucattelli M, Lunghi B, Martorana PA, *et al*. Different lung responses to cigarette smoke in two strains of mice sensitive to oxidants. Eur Respir J 2005;25:15–22. doi: 10.1183/09031936.04.00067204.
36. Villinger F, Brar SS, Mayne A, Chikkala N, Ansari AA. Comparative sequence analysis of cytokine genes from human and nonhuman primates. J Immunol 1995;155:3946–3954.
37. Helen W, Johnson JL, Jackson CL, White SJ, George SJ. MMP-7 mediates cleavage of N-cadherin and promotes smooth muscle cell apoptosis. Cardiovasc Res 2010;87:137–146. doi: 10.1093/cvr/cvq042.
38. DeSesso JM, Jacobson CF. Anatomical and physiological parameters affecting gastrointestinal absorption in humans and rats. Food Chem Toxicol 2001;39:209–228. doi: 10.1016/s0278-6915(00)00136-8.
39. Genomics Pennisi E. Sequence tells mouse, human genome secrets. Science 2002;298:1863–1865. doi: 10.1126/science.298.5600.1863.
40. Costa CH, Rufino R, Lapa ESJR. Inflammatory cells and their mediators in COPD pathogenesis. Rev Assoc Med Bras 19922009;55:347–354. doi: 10.1590/S0104-42302009000300031.
41. Kanazawa H, Asai K, Hirata K, Yoshikawa J. Possible effects of vascular endothelial growth factor in the pathogenesis of chronic obstructive pulmonary disease. Am J Med 2003;114:354–358. doi: 10.1016/S0002-9343(02)01562-0.
42. Betsuyaku T. Oxidative stress in pathogenesis of COPD. Nihon Rinsho 2007;65:633–636.
43. Valli G, Fedeli A, Antonucci R, Paoletti P, Palange P. Water and sodium imbalance in COPD patients. Monaldi Arch Chest Dis 2004;61:112–116. doi: 10.4081/monaldi.2004.708.
44. Cai S, Chen P, Zhang C, Chen JB, Wu J. Oral N-acetylcysteine attenuates pulmonary emphysema and alveolar septal cell apoptosis in smoking-induced COPD in rats. Respirology 2009;14:354–359. doi: 10.1111/j.1440-1843.2009.01511.x.
45. Wang B, Cleary PP, Xu H, Li JD. Up-regulation of interleukin-8 by novel small cytoplasmic molecules of nontypeable Haemophilus influenzae via p38 and extracellular signal-regulated kinase pathways. Infect Immun 2003;71:5523–5530. doi: 10.1128/iai.71.10.5523-5530.2003.
46. Birrell MA, Wong S, Hele DJ, McCluskie K, Hardaker E, Belvisi MG. Steroid-resistant inflammation in a rat model of chronic obstructive pulmonary disease is associated with a lack of nuclear factor-kappaB pathway activation. Am J Respir Crit Care Med 2005;172:74–84. doi: 10.1164/rccm.200409-1257OC.
47. Urboniene D, Sakalauskas R, Sitkauskiene B. C-reactive protein levels in patients with chronic obstructive pulmonary disease and asthma. Medicina (Kaunas) 2008;44:833–840. doi: 10.1159/000151576.
48. Tetley TD. Macrophages and the pathogenesis of COPD. Chest 2002;121:156s–159s. doi: 10.1378/chest.121.5_suppl.156s.
49. Undem BJ, Kollarik M. The role of vagal afferent nerves in chronic obstructive pulmonary disease. Proc Am Thorac Soc 2005;2:355–360. discussion 371-352. doi: 10.1513/pats.200504-033SR.
50. On LS, Boonyongsunchai P, Webb S, Davies L, Calverley PM, Costello RW. Function of pulmonary neuronal M(2) muscarinic receptors in stable chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2001;163:1320–1325. doi: 10.1164/ajrccm.163.6.2002129.
51. Longhini-Dos-Santos N, Barbosa-De-Oliveira VA, Kozma RH, Faria CA, Stessuk T, Frei F, *et al*. Cell therapy with bone marrow mononuclear cells in elastase-induced pulmonary emphysema. Stem Cell Rev 2013;9:210–218. doi: 10.1007/s12015-012-9419-y.
52. Busch RH, Lauhala KE, Loscutoff SM, Mcdonald KE. Experimental pulmonary emphysema induced in the rat by intratracheally administered elastase: morphogenesis. Environ Res 1984;33:497–513. doi: 10.1016/0013-9351(84)90044-6.
53. Abboud RT, Vimalanathan S. Pathogenesis of COPD. Part I. The role of protease-antiprotease imbalance in emphysema. Int J Tuberc Lung Dis 2008;12:361–367. doi: 10.1258/ijsa.2008.008015.
54. Herget J, Palecek F, Cermáková M, Vízek M. Pulmonary hypertension in rats with papain emphysema. Respiration 1979;38:204–212. doi: 10.1159/000194082.
55. Baila B, Ohno Y, Nagamoto H, Kotosai K, Yabuuchi Y, Funaguchi N, *et al*. Tetomilast attenuates elastase-induced pulmonary emphysema through inhibition of oxidative stress in rabbits. Biol Pharm Bull 2012;35:494–502. doi: 10.1248/bpb.35.494.
56. Lucas SD, Gonçalves LM, Cardote TAF, Correia HF, Rui M, Guedes RC. Structure based virtual screening for discovery of novel human neutrophil elastase inhibitors. Med Chem Comm 2012;3:1299–1304. doi: 10.1039/c2md20090b.
57. Milne J, Brand S. Occupational asthma after inhalation of dust of the proteolytic enzyme, papain. Br J Ind Med 1975;32:302–307. doi: 10.1136/oem.32.4.302.
58. Campbell EJ. Animal models of emphysema: the next generations. J Clin Invest 2000;106:1445–1446. doi: 10.1172/JCI11791.
59. Boyd RL, Fisher MJ, Jaeger MJ. Non-invasive lung function tests in rats with progressive papain-induced emphysema. Respir Physiol 1980;40:181–190. doi: 10.1016/0034-5687(80)90091-2.
60. Sulkowski S, Chyczewski L, Dziecioł J, Sulkowska M, Kozielec Z. Blood platelets in experimental lung emphysema. Comparative analysis of the number and aggregation abilities of platelets in left and right ventricular blood of the heart. Pol J Pathol 1994;45:299–302.
61. Sahebjami H, Vassallo CL. Influence of starvation on enzyme-induced emphysema. J Appl Physiol Respir Environ Exerc physiol 1980;48:284–288. doi: 10.1152/jappl.1980.48.2.284.
62. Edwards PD, Bernstein PR. Synthetic inhibitors of elastase. Med Res Rev 2010;14:127–194. doi: 10.1002/med.2610140202.
63. Raub JA, Mercer RR, Miller FJ, Graham JA, O'Neil JJ. Dose response of elastase-induced emphysema in hamsters. Am Rev Respir Dis 1982;125:432–435. doi: 10.1164/arrd.1982.125.4.432.
64. Mannino DM, Buist AS. Global burden of COPD: risk factors, prevalence, and future trends. Lancet 2007;370:765–773. doi: 10.1016/s0140-6736(07)61380-4.
65. Golovatch P. Smoking and High-Fat Diet: Risk Factors Regulating Emphysema Formation. Biology 2011. Available from: https://academiccommons.columbia.edu/doi/10.7916/D8RJ4RHQ/download. [Accessed July 25, 2019].
66. Wright JL, Churg A. Cigarette smoke causes physiologic and morphologic changes of emphysema in the guinea pig. Am Rev of Respir Dis 1990;142:1422–1428. doi: 10.1164/ajrccm/142.6_Pt_1.1422.
67. Gerrit J, Katrin K, Jürgen O, Ahmed R, Jürgen SK, Ralf Z, *et al*. The composition of cigarette smoke determines inflammatory cell recruitment to the lung in COPD mouse models. Clin Sci 2014;126:207–221. doi: 10.1042/cs20130117.
68. Shore S, Kobzik L, Long NC, Skornik W, Staden CJ, Van Boulet L, *et al*. Increased airway responsiveness to inhaled methacholine in a rat model of chronic bronchitis. Am J Respir Crit Care Med 1995;151:1931–1938. doi: 10.1164/ajrccm.151.6.7767542.
69. Leberl M, Kratzer A, Taraseviciene-Stewart L. Tobacco smoke induced COPD/emphysema in the animal model-are we all on the same page? Front Physiol 2013;4:91. doi: 10.3389/fphys.2013.00091.
70. van der Strate BW, Postma DS, Brandsma CA, Melgert BN, Luinge MA, Geerlings M, *et al*. Cigarette smoke-induced emphysema: a role for the B cell? Am J Respir Crit Care Med 2006;173:751–758. doi: 10.1164/rccm.200504-594OC.
71. Churg A, Cosio M, Wright JL. Mechanisms of cigarette smoke-induced COPD: insights from animal models. Am J Physiol Lung Cell Mol Physiol 2008;294:L612–631. doi: 10.1152/ajplung.00390.2007.

72. Valenca SS, da Hora K, Castro P, Moraes VG, Carvalho L, Porto LC. Emphysema and metalloelastase expression in mouse lung induced by cigarette smoke. Toxicol Pathol 2004;32:351–356. doi: 10.1080/01926230490431466.
73. He ZH, Chen P, Chen Y, He SD, Ye JR, Zhang HL, *et al*. Comparison between cigarette smoke-induced emphysema and cigarette smoke extract-induced emphysema. Tob Induc Dis 2015;13:6. doi: 10.1186/s12971-015-0033-z.
74. Eltom S, Stevenson C, Birrell MA. Cigarette smoke exposure as a model of inflammation associated with COPD. Curr Protoc Pharmacol 2013. Chapter 5 (Chapter 5): Unit 5.64. doi: 10.1002/0471141755.ph0564s60.
75. Pera T, Zuidhof AB, Smit M, Menzen MH, Klein T, Flik G, *et al*. Arginase inhibition prevents inflammation and remodeling in a guinea pig model of chronic obstructive pulmonary disease. J Pharmacol Exp Ther 2014;349:229–238. doi: 10.1124/jpet.113.210138.
76. Tazaki G, Kondo T, Tajiri S, Tsuji C, Shioya S, Tanigaki T. Functional residual capacity and airway resistance in rats of COPD model induced by systemic hyaluronidase. Tokai J Exp Clin Med 2006;31:125–127.
77. Misaka S, Sato H, Yamauchi Y, Onoue S, Yamada S. Novel dry powder formulation of ovalbumin for development of COPD-like animal model: Physicochemical characterization and biomarker profiling in rats. Eur J Pharm Sci 2009;37:469–476. doi: 10.1016/j.ejps.2009.04.002.
78. Wegmann M, Fehrenbach A, Heimann S, Fehrenbach H, Renz H, Garn H, *et al*. NO2-induced airway inflammation is associated with progressive airflow limitation and development of emphysema-like lesions in C57bl/6 mice. Exp Toxicol Pathol 2005;56:341–350. doi: 10.1016/j.etp.2004.12.004.
79. Gupta V, Banyard A, Mullan A, Sriskantharajah S, Southworth T, Singh D. Characterisation of the inflammatory response to inhaled LPS in mild to moderate COPD. Br J Clin Pharmacol 2014;79:767–776. doi: 10.1111/bcp.12546.
80. Taraseviciene-Stewart L, Douglas IS, Nana-Sinkam PS, Lee JD, Tuder RM, Nicolls MR, *et al*. Is alveolar destruction and emphysema in chronic obstructive pulmonary disease an immune disease? Proc Am Thorac Soc 2006;3:687–690. doi: 10.1513/pats.200605-105SF.
81. Chen Y, Chen P, Masayuki H, Peng H, Yunden D, Keishi K. The mechanism and pulmonary-protective effects of endothelin-1 receptor antagonist in chronic obstructive pulmonary diseases rat model (in Chinese). Chin J Intern Med 2010;49:380–384. doi: 10.3760/cma.j.issn.0578-1426.2010.05.005.
82. Zhang H, Chen P, Zeng H, Zhang Y, Peng H, Chen Y, *et al*. Protective effect of demethylation treatment on cigarette smoke extract-induced mouse emphysema model. J Pharmacol Sci 2013;123:159–166. doi: 10.1254/jphs.13072fp.
83. Yan Z, Yan C, Ping C, Hong P, Shan C, Hong L, *et al*. Intraperitoneal injection of cigarette smoke extract induced emphysema, and injury of cardiac and skeletal muscles in BALB/C mice. Exp lung Res 2013;39:18–31. doi: 10.3109/01902148.2012.745910.
84. Chen Y, Hanaoka M, Chen P, Droma Y, Voelkel NF, Kubo K. Protective effect of beraprost sodium, a stable prostacyclin analog, in the development of cigarette smoke extract-induced emphysema. Am J Physiol Lung Cell Mol Physiol 2009;296:L648–656. doi: 10.1152/ajplung.90270.2008.
85. He ZH, Chen Y, Chen P, He SD, Ye JR, Liu D. Decitabine enhances stem cell antigen-1 expression in cigarette smoke extract-induced emphysema in animal model. Exp Biol Med 2016;241:131–139. doi: 10.1177/1535370215598402.
86. He Z, Yan C, Hou C, He W, Ping C. Cigarette smoke extract changes expression of endothelial nitric oxide synthase (eNOS) and p16(INK4a) and is related to endothelial progenitor cell dysfunction. Med Sci Monit 2017;23:3224–3231. doi: 10.12659/MSM.902746.
87. Keil M, Lungarella G, Cavarra E, van Even P, Martorana PA. A scanning electron microscopic investigation of genetic emphysema in tight-skin, pallid, and beige mice, three different C57 BL/6J mutants. Lab Investi 1996;74:353–362. doi: 10.1121/1.414638.
88. Tirumalai R, Cho CY, Thimmulappa RK, Lijie Z, Srisuma SS, Kensler TW, *et al*. Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke-induced emphysema in mice. J Clin Invest 2004;114:1248–1259. doi: 10.1172/JCI21146.
89. Wert SE, Yoshida M, Levine AM, Ikegami M, Jones T, Ross GF, *et al*. Increased metalloproteinase activity, oxidant production, and emphysema in surfactant protein D gene-inactivated mice. Proc Natl Acad Sci U S A 2000;97:5972–5977. doi: 10.1073/pnas.100448997.
90. Morris DG, Xiaozhu H, Naftali K, Yanli W, Shapiro SD, Gregory D, *et al*. Loss of integrin alpha(v)beta6-mediated TGF-beta activation causes Mmp12-dependent emphysema. Nature 2003;422:169–173. doi: 10.1038/nature01413.
91. Baron RM, Choi AJ, Owen CA, Choi AM. Genetically manipulated mouse models of lung disease: potential and pitfalls. Am J Physiol Lung Cell Mol Physiol 2012;302:L485–497. doi: 10.1152/ajplung.00085.2011.
92. Liang R, Jin SD, Zhang X, Liu LJ, Rong HF. Abhd2 Genes and Emphysema Pathogenesis Research [in Chinese]. Progr Modern Biomedicine 2011;11:4430–4433.
93. Wan ES, Silverman EK. Genetics of COPD and Emphysema. Chest 2009;136:859–866. doi: 10.1378/chest.09-0555.
94. Su B, Liu T, Fan H, Chen F, Ding H, Wu Z, *et al*. Inflammatory markers and the risk of chronic obstructive pulmonary disease: a systematic review and meta-analysis. PLoS One 2016;11:e0150586. doi: 10.1371/journal.pone.0150586.
95. Suga T, Kurabayashi M, Sando Y, Ohyama Y, Maeno T, Maeno Y, *et al*. Disruption of the klotho gene causes pulmonary emphysema in mice. Defect in maintenance of pulmonary integrity during postnatal life. Am J Respir Cell Mol Biol 2000;22:26–33. doi: 10.1165/ajrcmb.22.1.3554.
96. Kazama K, Usui T, Okada M, Hara Y, Yamawaki H. Omentin plays an anti-inflammatory role through inhibition of TNF-(-induced superoxide production in vascular smooth muscle cells. Eur J Pharmacol 2012;686:116–123. doi: 10.1016/j.ejphar.2012.04.033.
97. Hoyle GW, Li J, Finkelstein JB, Eisenberg T, Liu JY, Lasky JA, *et al*. Emphysematous lesions, inflammation, and fibrosis in the lungs of transgenic mice overexpressing platelet-derived growth factor. Am J Pathol 1999;154:1763–1775. doi: 10.1016/s0002-9440(10)65432-6.
98. Dalal S, Imai K, Mercer B, Okada Y, Chada K, D'Armiento JM. A role for collagenase (Matrix metalloproteinase-1) in pulmonary emphysema. Chest 2000;117:227s–228s. doi: 10.1378/chest.117.5_suppl_1.227s.
99. Wallace AM, Loy LB, Abboud RT, D'Armiento JM, Coxson HO, Muller NL, *et al*. Expression of Matrix Metalloproteinase-1 in Alveolar Macrophages, Type II pneumocytes, and airways in smokers: relationship to lung function and emphysema. Lung 2014;192:467–472. doi: 10.1007/s00408-014-9585-6.
100. Mackay AJ, Hurst JR. COPD exacerbations: causes, prevention, and treatment. Immunol Allergy Clin North Am 2013;33:95–115. doi: 10.1016/j.iac.2012.10.006.
101. Nie YC, Wu H, Li PB, Luo YL, Zhang CC, Shen JG, *et al*. Characteristic comparison of three rat models induced by cigarette smoke or combined with LPS: to establish a suitable model for study of airway mucus hypersecretion in chronic obstructive pulmonary disease. Pulm Pharmacol Ther 2012;25:349–356. doi: 10.1016/j.pupt.2012.06.004.
102. Malcolm Ronald S, Andrew Gregory J, Ama-Tawiah E, Shaan Lae G, Richard Yong K, Alexandra Cerelina B, *et al*. Murine models of infectious exacerbations of airway inflammation. Curr Opin Pharmacol 2013;13:337–344. doi: 10.1016/j.coph.2013.03.005.
103. Donovan C, Seow HJ, Bourke JE, Vlahos R. Influenza A virus infection and cigarette smoke impair bronchodilator responsiveness to beta-adrenoceptor agonists in mouse lung. Clin Sci (Lond) 2016;130:829–837. doi: 10.1042/cs20160093.
104. Stolk J, Rudolphus A, Davies P, Osinga D, Dijkman JH, Agarwal L, *et al*. Induction of emphysema and bronchial mucus cell hyperplasia by intratracheal instillation of lipopolysaccharide in the hamster. J Pathol 1992;167:349–356. doi: 10.1002/path.1711670314.
105. Huvenne W, Lanckacker EA, Krysko O, Bracke KR, Demoor T, Hellings PW, *et al*. Exacerbation of cigarette smoke-induced pulmonary inflammation by Staphylococcus aureus enterotoxin B in mice. Respir Res 2011;12:69. doi: 10.1186/1465-9921-12-69.
106. Kaminsky DA. What does airway resistance tell us about lung function? Respir Care 2012;57:85–96. discussion 96-89. doi: 10.4187/respcare.01411.
107. He ZH, Chen Y, Chen P, He SD, Zeng HH, Ye JR, *et al*. 5-Aza-2′-deoxycytidine protects against emphysema in mice via suppressing

p16Ink4a expression in lung tissue. Int J Chron Obstruct Pulmon Dis 2017;12:3149–3158. doi: 10.2147/COPD.S131090.
108. Tang Y, Cai QH, Wang YJ, Fan SH, Zhang ZF, Xiao MQ, *et al*. Protective effect of autophagy on endoplasmic reticulum stress induced apoptosis of alveolar epithelial cells in rat models of COPD. Biosci Rep 2017;37. pii: BSR20170803. doi: 10.1042/bsr20170803.
109. Song Y, Yu P, Lu JJ, Lu HZ, Zhu L, Yu ZH, *et al*. A mucoactive drug carbocisteine ameliorates steroid resistance in rat COPD model. Pulm Pharmacol Ther 2016;39:38–47.
110. March TH, Wilder JA, Esparza DC, Cossey PY, Blair LF, Herrera LK, *et al*. Modulators of cigarette smoke-induced pulmonary emphysema in A/J mice. Toxicol Sci 2006;92:545–559. doi: 10.1093/toxsci/kfl016.
111. Schober A, Feiner JR, Bickler PE, Rollins MD. Effects of changes in arterial carbon dioxide and oxygen partial pressures on cerebral oximeter performance. Anesthesiology 2018;128:97–108. doi: 10.1097/aln.0000000000001898.
112. Vlahos R, Bozinovski S. Recent advances in pre-clinical mouse models of COPD. Clin Sci (Lond) 2014;126:253–265. doi: 10.1042/cs20130182.
113. Feizpour A, Boskabady MH, Ghorbani A. Adipose-derived stromal cell therapy affects lung inflammation and tracheal responsiveness in guinea pig model of COPD. PLoS One 2014;9:e108974. doi: 10.1371/journal.pone.0108974.
114. De Brauwer EI, Jacobs JA, Nieman F, Bruggeman CA, Drent M. Bronchoalveolar lavage fluid differential cell count. How many cells should be counted? Anal Quant Cytol Histol 2002;24:337–341.
115. Nakao A, Miike S, Hatano M, Okumura K, Tokuhisa T, Ra C, *et al*. Blockade of transforming growth factor beta/Smad signaling in T cells by overexpression of Smad7 enhances antigen-induced airway inflammation and airway reactivity. J Exp Med 2000;192:151–158. doi: 10.1084/jem.192.2.151.
116. Freiredelima CG, Yi QX, Gardai SJ, Bratton DL, Schiemann WP, Henson P. Apoptotic cells, through transforming growth factor-(, Coordinately induce anti-inflammatory and suppress pro-inflammatory eicosanoid and NO synthesis in murine macrophages. J Biol Chem 2006;281:38376–38384. doi: 10.1074/jbc.M605146200.
117. Zhang Q, Huang P, Li Y, Yao X, Sun Y, Wang W, *et al*. Evaluation of a mouse model of chronic obstructive pulmonary disease induced by intraperitoneal injections of cigarette smoke extract (in Chinese). Chin J Tubere Respir Dis 2015;38:279–285. doi: 10.3760/cma.j.issn.1001-0939.2015.04.010.
118. Wang XL, Li T, Li JH, Miao SY, Xiao XZ. The effects of resveratrol on inflammation and oxidative stress in a rat model of chronic obstructive pulmonary disease. Molecules 2017;22:E1529. doi: 10.3390/molecules22091529.
119. Wang Z, Yang W, Yang P, Gao B, Luo L. Effect of Radix Stemonae concentrated decoction on the lung tissue pathology and inflammatory mediators in COPD rats. BMC Complement Altern Med 2016;16:457. doi: 10.1186/s12906-016-1444-y.
120. Vlaykova T, Dimov D. Polymorphisms of Matrix Metalloproteinases (MMP) in COPD. Biotechnol Biotechnol Equip 2012;26:111–119. doi: 10.5504/50YRTIMB.2011.0021.
121. Elias JA, Kang MJ, Crothers K, Homer R, Lee CG. State of the art. Mechanistic heterogeneity in chronic obstructive pulmonary disease: insights from transgenic mice. Proc Am Thorac Soc 2006;3:494–498. doi: 10.1513/pats.200603-068MS.
122. Zhang K, Guo L, Wei Q, Song Q, Liu J, Niu J, *et al*. COPD rat model is more susceptible to cold stress and PM2.5 exposure and the underlying mechanism. Environ Pollut 2018;241:26–34. doi: 10.1016/j.envpol.2018.05.034.
123. Cao Y, Zhou X, Yin Z, Yu X, Yang Q, Guo Q, *et al*. The anti-inflammatory effect of BML-111 on COPD may be mediated by regulating NLRP3 inflammasome activation and ROS production. Prostaglandins Other lipid Mediat 2018;138:23–30. doi: 10.1016/j.prostaglandins.2018.08.001.
124. Yamada K, Asai K, Nagayasu F, Sato K, Ijiri N, Yoshii N, *et al*. Impaired nuclear factor erythroid 2-related factor 2 expression increases apoptosis of airway epithelial cells in patients with chronic obstructive pulmonary disease due to cigarette smoking. BMC Pulm Med 2016;16:27. doi: 10.1186/s12890-016-0189-1.
125. Wang Y, Jiang X, Zhang L, Wang L, Li Z, Sun W. Simvastatin mitigates functional and structural impairment of lung and right ventricle in a rat model of cigarette smoke-induced COPD. Int J Clin Exp Pathol 2014;7:8553–8562.
126. Zhao YL, Li F, Liu YW, Shi YJ, Li ZH, Cao GK, *et al*. Adiponectin attenuates endoplasmic reticulum stress and alveolar epithelial apoptosis in COPD rats. Eur Rev Med Pharmacol Sci 2017;21:4999–5007.
127. Westergrenthorsson G, Larsen K, Nihlberg K, Anderssonsjöland A, Hallgren O, Markovarga G, *et al*. Pathological airway remodelling in inflammation. Clin Respir J 2010;4:1–8. doi: 10.1111/j.1752-699X.2010.00190.x.
128. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. American Thoracic Society. Am J Respir Crit Care Med 1995;152:S77–121. doi: 10.1016/j.jhazmat.2008.11.047.
129. Donaldson GC, Seemungal TAR, Patel IS, Bhowmik A, Wilkinson TMA, Hurst JR, *et al*. Airway and systemic inflammation and decline in lung function in patients with COPD. Chest 2005;128:1995–2004. doi: 10.10.1378/chest.128.4.1995.
130. Wright JL, Cosio M, Churg A. Animal models of chronic obstructive pulmonary disease. Am J Physiol Lung Cell Mol Physiol 2008;295:L1–15. doi: 10.10.1152/ajplung.90200.2008.
131. Ochs M. Estimating structural alterations in animal models of lung emphysema. Is there a gold standard? Ann Anat 2014;196:26–33. doi: 10.10.1016/j.aanat.2013.10.004.

How to cite this article: Liang GB, He ZH. Animal models of emphysema. Chin Med J 2019;132:2465–2475. doi: 10.1097/CM9.0000000000000469