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Experimental Animal Models in Respiratory Diseases

Solunum Hastalıklarında Deneysel Hayvan Modelleri

Pınar YILDIZ GÜLHAN[1,2]
0000-0002-5347-2365

[1]Department of Chest Diseases, Düzce University Faculty of Medicine, Düzce, Türkiye
[2]Experimental Animals Application and Research Center, Düzce University, Düzce, Türkiye

Corresponding Author
Sorumlu Yazar
Pınar YILDIZ GÜLHAN
pinaryildiz691@hotmail.com

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ABSTRACT

Respiratory diseases are among the leading causes of morbidity and mortality worldwide. Various animal models are used to understand the pathogenesis of these diseases and develop novel therapeutic strategies. Each model offers the opportunity to examine the multifaceted nature of pulmonary health, from common afflictions such as asthma and chronic obstructive pulmonary disease (COPD) to interstitial lung diseases. While these models provide a unique opportunity to understand normal physiology and disease pathophysiology and to test potential treatments for diseases, all animal models have inherent limitations. This review focuses on experimental models of common respiratory diseases such as asthma, COPD, and pulmonary fibrosis. The advantages, disadvantages, and translational potential to human disease of each model are discussed. Asthma models include mice, guinea pigs, and Drosophila, while elastase-induced emphysema, cigarette smoke exposure, and genetically modified mice are used for COPD. For pulmonary fibrosis, bleomycin, adenoviral TGF-β1 vector, silica, and genetically modified mice models are available. These models have provided valuable insights into disease mechanisms and aided in identifying new therapeutic targets. However, it is important to note that no single model fully recapitulates human disease, and each has its own unique advantages and limitations. Therefore, careful consideration of the translatability of findings from preclinical studies to humans is crucial.
Keywords: Experimental animal; lung diseases; pathogenesis.

ÖZ

Akciğer hastalıkları, dünya genelinde morbidite ve mortalitenin önde gelen nedenlerindendir. Bu hastalıkların patogenezini anlamak ve yeni tedavi stratejileri geliştirmek için çeşitli hayvan modelleri kullanılmaktadır. Her model, astım ve kronik obstrüktif akciğer hastalığı (KOAH) gibi yaygın rahatsızlıklardan interstisyel akciğer hastalıklarına kadar akciğer sağlığının çok yönlü doğasını inceleme fırsatı sunar. Bu modeller normal fizyolojiyi ve hastalık patofizyolojisini anlamak ve hastalıklara yönelik potansiyel tedavileri test etmek için eşsiz bir fırsat sağlarken, tüm hayvan modellerinin doğası gereği sınırlamaları vardır. Bu derlemede, astım, KOAH ve pulmoner fibroz gibi yaygın akciğer hastalıklarının deneysel modellerine odaklanılmıştır. Her modelin avantajları, dezavantajları ve insan hastalığına translasyonel potansiyeli tartışılmaktadır. Astım modelleri arasında fareler, kobaylar ve Drosophila bulunurken, KOAH için elastazla indüklenen amfizem, sigara dumanına maruziyet ve genetik olarak değiştirilmiş fareler kullanılmaktadır. Pulmoner fibroz için ise bleomisin, adenoviral TGF-β1 vektörü, silika ve genetik olarak değiştirilmiş fare modelleri mevcuttur. Bu modeller, hastalık mekanizmalarına dair değerli bilgiler sağlamış ve yeni terapötik hedeflerin belirlenmesine yardımcı olmuştur. Bununla birlikte, her modelin insan hastalığını tam olarak yansıtmadığı ve her birinin kendine özgü avantajları ve sınırlamaları olduğu unutulmamalıdır. Bu nedenle, klinik öncesi çalışmalarda elde edilen bulguların insanlara uygulanabilirliğini dikkatlice değerlendirmek önemlidir.
Anahtar kelimeler: Akciğer hastalıkları; deney hayvanı; patogenez.

INTRODUCTION

Animal models have been instrumental in investigating normal physiology, the pathophysiology of diseases, and testing the safety and efficacy of novel therapeutics in preclinical studies (1). From commonly encountered afflictions such as asthma and chronic obstructive pulmonary disease (COPD) to the more nuanced realms of interstitial lung diseases, each model offers a unique lens through which we can examine the multifaceted nature of pulmonary health. While these models provide a unique opportunity to understand normal physiology, and disease pathophysiology, and test potential therapies for diseases, all animal models inherently possess limitations (2). The first limitation is that the genetic and physiological differences between experimental animals and humans can lead to an incomplete replication of human diseases. For instance, the lung structure and immune system of mice differ from those of humans. Therefore, lung disease models created in mice may not reflect all the characteristics of the disease in humans. The second limitation is that lung disease models created in experimental animals may not fully reflect the progression and severity of the disease. In humans, lung diseases often develop over a long period, whereas in experimental animals, this process can be accelerated. This can affect the natural course of the disease and the response to treatment. In addition to these limitations, ethical concerns and the welfare of experimental animals should also be considered. The use of experimental animals should be reduced as much as possible and alternative methods should be investigated (3).

In this review, it was aimed to write about different experimental models of respiratory diseases aiming to unravel the complex mechanisms underlying their pathophysiology.

ASTHMA MODEL

Asthma, a global health issue affecting an estimated 300 million people across all ages, is characterized by fluctuating respiratory symptoms like wheezing, shortness of breath, chest tightness, and cough, alongside varying limitations in exhaling air. The condition's prevalence is on the rise. This chronic inflammatory disease causes structural changes in the airways, including increased mucus production, tissue scarring, abnormal cell growth, and enlarged airway muscles (4,5). Animal models play a crucial role in deciphering the underlying disease mechanisms and assessing the safety and effectiveness of potential new therapies before they are tested in humans (6).

Various animal species have been utilized in experimental asthma models, including Drosophila, rats, guinea pigs, cats, dogs, pigs, primates, and horses. However, in the last two decades, the most commonly studied species have been mice, particularly BALB/c mice (7).

Drosophila Melanogaster

The respiratory organ of the fruit fly *D. melanogaster* is the tracheal system, a network of epithelial tubes that branch throughout the body, carrying gases to tissues and organs. This network takes in gases from respiratory openings called spiracles, which provide an external connection to the system, and transports them to tissues and organs through tracheal branches that branch out in the body (8).

In particular, the fruit fly *Drosophila* has been successfully positioned as a genetically tractable model for studying the molecular architecture underlying various chronic lung diseases, such as asthma, COPD, and lung cancer. Although insects share surprising commonalities with human lungs in terms of structure, physiology, organogenesis, and innate immune system, they have a very simple, entirely epithelial airway system (9).

Guinea Pig

Guinea pigs do not naturally have asthma; however, they are known to exhibit immediate hypersensitivity reactions in their lungs (10). Ovalbumin (OVA) is commonly used as a sensitizer in inducible animal models (such as mice and rats) of T2-heavy asthma. Guinea pigs can also be sensitized to OVA or other stimuli, triggering IgE-mediated mechanisms similar to the human asthma phenotype, with eosinophilia and increased airway responsiveness (11).

OVA can be administered through various routes, including peritoneal, subcutaneous, and aerosol forms. Low-dose OVA (10 μg) can induce early asthmatic responses (EARs) with IgE and IgG1 production (12-13). Higher doses (100 μg) can trigger both early and late asthmatic responses (LARs) (11).

In asthmatic humans, acute allergic hypersensitivity reactions in the airways (airway smooth muscle contraction, eosinophil infiltration, airway hyperresponsiveness, and mucus production) are partially mediated by activation of histamine H1 and cysteinyl leukotriene (cysLT)-1 receptors. These receptor activations have also been observed in guinea pigs (14). Due to these similarities, guinea pigs have played a significant role in the development of asthma treatments; for example, leukotriene receptor antagonists (such as Montelukast) and phosphodiesterase (PDE3/4) inhibitors (such as ensifentrine) have been developed in these models (3,11).

Despite their benefits, guinea pigs have some limitations as an asthma model. They have obligate nasal breathing due to their long soft palate, possess seven lung lobes with a specific branching pattern, and their lung parenchyma is more fragile than that of humans. Chronic asthma is generally not inducible in guinea pigs, as they develop tolerance to allergens and do not exhibit non-specific hyperresponsiveness. Additionally, bronchoconstriction in guinea pigs is mostly mediated by histamine, which limits the translational value for human asthma since antihistamines have limited efficacy in humans. Other challenges include a longer gestation period and limited availability of reactive and transgenic lines. Efforts are ongoing to develop more assays, identify translatable markers, and increase the availability of monoclonal antibodies to make research in guinea pigs more feasible (11,15).

Mice Models

Mice, not prone to developing asthma naturally, require artificial induction of asthma-like reactions for research purposes. Acute allergic responses to inhaled allergens in mice are commonly studied to understand the immunological and inflammatory mechanisms underlying asthma and identify novel targets for managing allergic inflammation. Various allergens, such as OVA, house dust mites, fungal extracts, and cockroach extracts, are utilized depending on the specific condition being replicated (16).

Allergens used in animal models are OVA, house dust mites such as *Dermatophagoides pteronyssinus* (*Der p*) or *D. farinae* (*Der f*), mite allergens (Der p 1, Der f 1, Der p 23, etc.), fungi (*Aspergillus fumigatus*, *Alternaria alternata*), cockroach extracts, Ascaris antigens, cotton dust, ragweed and latex (*Hevea brasiliensis*). The allergen selected depends on the condition to be replicated and can be used individually or in combination (17).

BALB/c mice, known for their tendency to develop a strong T helper cell 2 (Th2)-biased immune response, are the most frequently used strain in antigen-challenge models (18). However, other strains like C57BL/6 and A/J have also been employed successfully (19). OVA, derived from chicken egg, is a widely used allergen that effectively induces allergic lung inflammation in laboratory animals.

Mouse models for allergic asthma involve sensitizing the animal to a foreign protein, typically OVA, through intraperitoneal injections with an adjuvant to enhance immunogenicity (20). Following sensitization, the animal is challenged with further antigen exposure through aerosol inhalation or nasal drip, triggering an inflammatory response in the lungs. This response is characterized by eosinophil influx, epithelial thickening, and airway hyperresponsiveness. The specific methods used for sensitization and challenge can vary among investigators, but a typical approach involves two-spaced intraperitoneal injections followed by a week of rest and then daily exposure to 1% OVA aerosol for three days (21). Allergic inflammation typically peaks one or two days after the final challenge, although the precise time course of this process remains incompletely understood.

Chronic allergen challenge models expose the airways to low allergen levels repeatedly for extended periods, up to 12 weeks. These models often utilize various allergens, including OVA, house dust mite extract, or grass pollen, without always requiring adjuvant co-administration. They simulate long-term allergen exposure and its effects on airway inflammation and remodeling (22-24).

While mouse allergen challenge models are invaluable in asthma research, the complex and diverse nature of asthma makes it unlikely that any single model can fully recapitulate the clinical disease. Therefore, studies focus on modeling specific asthma phenotypes rather than attempting to replicate all features of asthma in a single model. This approach allows for targeted investigation of particular disease mechanisms and potential therapeutic interventions.

- Acute Allergen Challenge Models: These are commonly used to investigate lung inflammation and airway hyperresponsiveness. However, they have limitations, particularly in relating findings to chronic asthma.
- Chronic Allergen Challenge Models: These appear to be able to reproduce some features of chronic asthma. This allows them to address fundamental questions regarding both the pathogenesis of asthma and new therapeutic approaches.

Both acute and chronic allergen challenge models have limitations that need to be considered when integrating findings from the animal model into human disease. These limitations include:

- The lung structure of mice differs from that of humans.
- The mouse immune system responds differently from the human immune system.
- Mouse models may not fully reflect environmental factors.

Therefore, while mouse allergen challenge models are an important tool in asthma research, careful consideration must be given to how the results translate to human disease (16).

CHRONIC OBSTRUCTIVE PULMONARY DISEASE MODEL

COPD is a debilitating, progressive lung disease characterized by breathing difficulties, chronic inflammation, and tissue breakdown. Due to ethical considerations, animal models are indispensable in COPD research involving human subjects. These models enhance our understanding of COPD's underlying mechanisms, including its physiology, pathophysiology, and treatment responses. While not perfectly replicating all aspects of the human disease, animal models provide valuable insights into the processes involved in COPD (25).

Various approaches exist to simulate COPD in animal models. These include exposing animals to cigarette smoke (the primary cause of COPD), inflammatory stimuli like lipopolysaccharide, proteolytic enzymes such as elastase, and genetic modifications. The choice of model depends on the specific research goals (26-28).

The subsequent discussion will delve into the diverse models employed in COPD research.

Elastase-Induced Animal Model of Emphysema

Since the 1970s, the role of elevated protein breakdown in COPD's development has garnered significant research interest. In COPD patients, an imbalance exists between protein-degrading elastases (MMP family) and anti-elastases in lung tissues. Excessive elastase release from inflammatory cells can damage the lung parenchyma, leading to emphysema. Notably, genetic alpha-1-antitrypsin deficiency, which disrupts the elastase-antielastase balance, has been directly linked to emphysema-like changes (29,30).

To model emphysema, a simple and cost-effective method involves instilling elastase drops into the trachea. This approach disrupts the protease-antiprotease balance in lung tissue, both removing protective factors and generating numerous inflammatory factors, thereby accelerating alveolar wall rupture and fusion, ultimately leading to emphysema. Commonly used elastases in this model include:

Papain

A plant-derived proteolytic enzyme and the first elastase used to create an emphysema model. In the 1960s, a successful rat emphysema model was first created using papain. Although the use of different doses of papain was tried in subsequent studies, no significant difference was found between doses. Therefore, the instillation of 2 mg/kg papain into the trachea in a single dose has been accepted as a relatively suitable method (31).

Porcine Pancreatic Elastase (PPE)

Commonly used to create emphysema models in animals, PPE is obtained from porcine pancreas. PPE not only acts as a protease, disrupting the protease-antiprotease balance but also acts as an oxidant, causing oxidative stress.

Thanks to this dual effect, the alveoli expand significantly in the experimental animal model. Therefore, PPE is often used to create emphysema. Usage doses vary between 6 and 24 U. Methods for creating emphysema with PPE generally include intratracheal instillation, tracheotomy injection, and atomizer inhalation. It usually takes 4 to 6 weeks to create emphysema-like changes with these methods (30,32).

Human Neutrophil Elastase (HNE)

A serine protease that plays an important role in the inflammatory process of COPD. Protease/anti-protease imbalance causes excessive hydrolysis of elastin and structural proteins that give elasticity to lung tissue by extracellular HNE. Since HNE's ability to enter the alveolar septum and break down elastic fibers is weak, it is rarely used to create emphysema today (33).

While papain is the earliest method used, PPE is more commonly preferred due to its ease of use and effectiveness. HNE is not preferred due to its weak efficacy (30)

Emphysema Model Creation with Cigarette Smoke Exposure

In the realm of COPD research, cigarette smoke exposure holds significant importance, as approximately 90% of COPD patients are smokers. Cigarette smoke exposure is a major risk factor for emphysema, and animal models employing cigarette smoke exposure have been instrumental in understanding its development.

In 1990, Wright et al. (34) first succeeded in creating a guinea pig emphysema model through cigarette smoke exposure. They found that long-term smoking caused changes in the center of the lobules, creating emphysema, as in humans. Long-term cigarette smoke exposure in animals can cause an inflammatory response in the lungs, mostly consisting of macrophages (35). As a result, the bronchial lumen narrows and the bronchial cartilage tissue is damaged. This leads to alveolar rupture, fusion, and emphysema formation, just as in humans exposed to cigarette smoke. Passive smoking-induced emphysema can mimic the pathogenesis of human emphysema as closely as possible and provide a basis for basic and clinical research on human emphysema.

The airway and lung structures of experimental animals differ between species and from humans. Guinea pigs are among the most sensitive animals to cigarette smoke. Rats, on the other hand, show some resistance to cigarette smoke, but there are also differences in sensitivity between different rat species. The experimental duration for a passive smoking-induced COPD animal model is relatively long and its stability is also relatively low (36).

Cigarette smoke exposure can be broadly classified in two ways; the first method is the partial exposure (nose or head only) method. van der Strate et al. (37) studied C57BL/6J mice that inhaled cigarette smoke through their noses twice a day, 2 cigarettes each time, 10 puffs per cigarette. The results showed that pulmonary alveoli expanded with increasing exposure time. At the same time, B lymphocytes in the lung tissues of smoking mice increased, similar to those seen in human emphysema.

The other method is the whole-body exposure method (38). In this method, the experimental animal is placed in a box completely filled with smoke. Valenca et al. (39) exposed C57BL/6 mice to cigarette smoke 3 times a day, 3 cigarettes each time. After 60 days, emphysema-like changes were observed in the lungs, with increased alveolar macrophages, extracellular matrix changes, and increased MMP-12 expression.

The passive smoking method is quite popular due to its low cost, simple application, high success rate, and elimination of experimental differences in a more objective environment. The duration of cigarette smoke exposure in the experimental emphysema model may vary depending on various factors such as the type of cigarette used, the method of exposure, duration and frequency, smoke density, and the species and age of the animals.

Animal Models of Chemical-Induced Emphysema

Many chemicals common in air pollution, such as nitric oxide (NO_2), can cause emphysema and inflammation in animals. These chemicals include:

- Nitric Oxide (NO_2)
- Lipopolysaccharide (LPS)
- Ozone (O_3)
- Cadmium chloride ($CdCl_2$)
- Hyaluronidase enzyme (intravenous injection)
- Ovalbumin dust (inhalation)

Nitric Oxide (NO_2)

In an experimental setting, emphysema can be induced in animals by controlling the NO_2 concentration and inhalation duration. Wegmann et al. (40) observed that long-term exposure of mice to 20 x 106 volume ratio NO_2 for 14 hours a day for 25 days resulted in emphysema due to oxidative stress.

Lipopolysaccharide (LPS)

Lipopolysaccharide primarily causes inflammation in airway and lung tissue by stimulating neutrophils, monocytes, and endothelial cells. These cells secrete a series of inflammatory mediators such as TNF-α, IL-1, and trigger protease-antiprotease imbalance, leading to emphysema (41).

Cadmium Chloride ($CdCl_2$)

Snider et al. (42) created an animal model of emphysema by instilling 0.5 ml of 0.025% $CdCl_2$ solution into the trachea of golden-mantled ground squirrels in a single dose.

Gene-Knockout Animal Models of Emphysema

Advancements in molecular biology and the Human Genome Project have enabled scientists to explore the intricate relationship between genes and diseases, leading to the development of emphysema animal models through genetic manipulation. By editing genes related to emphysema, researchers hope to uncover new insights into this complex condition.

Spontaneous emphysema, initially discovered in spotted mice in the 1970s, has been linked to abnormal connective tissue mechanisms and cross-linking of collagen and elastin. Long-term animal experiments have identified spontaneous emphysema in various mouse strains, including Tit-skin, Beige, Blotchy, and Palliad mice (43).

With the advancement of molecular biology, emphysema animal models created using gene knockout methods are widely utilized in emphysema research. In recent years, an increasing number of studies have employed gene knockout techniques to replicate animal models (30).

Liang et al. (44) found that mice with a knockout of the Abhd2 gene exhibited emphysema-like changes in the

lungs due to excessive inflammation cytokines and protease gene expression, increased macrophage numbers, abnormal apoptosis, and resistance to the deficiency or loss of protease inhibitors. The models showed a similar progressive emphysema development in formation, development process, and clinical pathology. Therefore, examining the genetic susceptibility and environmental factors of emphysema is of great importance.

ANIMAL MODELS FOR IDIOPATHIC PULMONARY FIBROSIS (IPF)

Idiopathic pulmonary fibrosis (IPF) is a chronic, incurable lung disease characterized by fibrosis (stiffness), inflammation, and tissue damage in the lungs (45). Damage to the alveolar epithelium and abnormal wound healing are key factors in the disease's progression. IPF typically affects individuals around 65 years old, with a 3-5 year survival rate post-diagnosis and an estimated annual incidence of 5.6 per 100,000 people (46).

While the exact causes of IPF remain elusive, a combination of genetic and environmental factors is suspected. Continuous micro-injuries to the aging alveolar epithelium disrupt epithelial-fibroblast communication, triggering myofibroblasts to produce and activate collagen-rich extracellular matrix. This excessive matrix accumulation leads to irreversible alveolar collapse, impairing gas exchange and making breathing difficult. In the absence of a cure, treatment focuses on slowing fibrosis progression, maintaining comfort, and providing palliative care in advanced stages (47,48).

Given the lack of curative options, further research using animal models that closely resemble human IPF is crucial for developing potential therapies. While spontaneous pulmonary fibrosis in domestic animals like cats and dogs offers some insights, rodent models remain essential for investigating disease pathogenesis and conducting preclinical therapeutic evaluations. A variety of established and emerging experimental models provide valuable information about disease mechanisms, aiding in the identification of novel therapeutic targets for clinical trials.

Bleomycin Model

Bleomycin, a chemotherapeutic antibiotic derived from the bacterium Streptomyces verticillatus, is utilized in cancer treatment but carries the risk of acute lung damage and fibrosis as adverse effects in humans (49). While effective against cancer, it can also induce scar tissue formation in the lungs.

The bleomycin model, an animal model employed to study lung fibrosis, presents a combination of strengths and weaknesses. Recognized by the ATS workshop as the most well-established model for preclinical testing, it offers researchers a reliable tool for obtaining consistent results (50). Bleomycin inflicts damage upon lung cells, swiftly triggering inflammation and fibrosis through an initial surge in proinflammatory cytokines, followed by an increase in profibrotic factors. The effects of fibrosis become evident within a week and peak in 3-4 weeks. However, a notable limitation is that bleomycin-induced fibrosis is transient and histopathologically distinct from the persistent fibrosis observed in human IPF (51).

Mouse responses to bleomycin exhibit genetic variations, mirroring the diversity seen in humans. For instance, C57Bl/6 mice demonstrate greater susceptibility compared to Balb/c mice, likely due to differences in cytokine and protease expression patterns (52). Most research utilizes young mice aged 8-12 weeks, corresponding to human puberty. Notably, male mice are more susceptible to bleomycin-induced lung injury than females, similar to the pattern observed in humans, although the underlying mechanisms remain incompletely understood (53).

Inhalation (intratracheal) is the most prevalent and well-established method of bleomycin administration, favored for its ease of use and ability to confine lung damage to the lungs, mimicking the human disease. However, alternative routes such as intraperitoneal, subcutaneous, and intravenous administration exist (50).

In the bleomycin model, interventions during the inflammatory phase predominantly exert anti-inflammatory effects and are considered "preventive treatment." To investigate the "therapeutic" effects of antifibrotic agents, intervention after the inflammatory period (usually after day 7) is recommended, highlighting the importance of optimizing treatment timing in this model. This recommendation aligns with those put forth by the European Respiratory Society Task Force for optimizing experimental models of lung diseases (Figure 1, 54).

Interestingly, Peng et al. (55) reported that repeated low-dose intratracheal bleomycin administration could potentially induce irreversible lung injury lasting 24 weeks. However, this approach is impractical for most studies due to high mortality rates, cost, and time constraints. Therefore, factors like administration route, intervention timing, and fibrosis persistence warrant careful consideration to maximize the effectiveness of the bleomycin model in developing treatments for pulmonary fibrosis.

Adenoviral TGF-β1 Vector Model

In this model, an adenovirus acts as a carrier to deliver the TGF-β1 gene to lung cells. TGF-β1, a protein pivotal in lung fibrosis, is produced by lung cells due to the introduced gene, ultimately leading to fibrosis development. Importantly, TGF-β1 plays a crucial role in IPF.

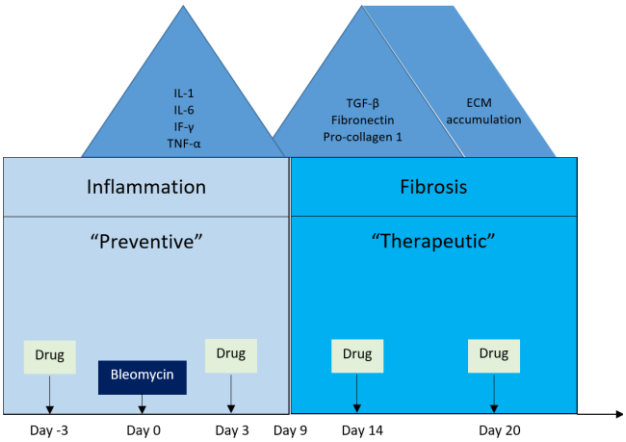

Figure 1. Bleomycin administration triggers an acute inflammatory response for up to 8 days, followed by fibrogenic changes and matrix deposition leading to lung structure distortion out to 28 or 35 days. Treatments within the initial 7 days are considered "preventive," while interventions after days 7-10 are deemed "therapeutic" (54).

The adenoviral TGF-β1 model offers a more accurate representation of the fibrosis environment compared to the bleomycin model. Unlike the bleomycin model, where fibrosis resolves over time, fibrosis in this model persists for an extended period, up to day 64, mirroring the persistent nature of fibrosis in IPF (56).

Intratracheal administration of AdTGF-β1 triggers high expression levels of activated TGF-β1, resulting in mild initial inflammation and rapid lung fibrosis. Notably, this model also induces pulmonary hypertension in rats, a common and severe complication in IPF patients with a poorer prognosis. Consequently, this model proves valuable for investigating the pathogenesis of pulmonary hypertension secondary to pulmonary fibrosis (57).

Genetically Modified Mouse Models of Lung Fibrosis

Genetic predisposition plays a significant role in the development of lung fibrosis. Mutations in genes such as surfactant protein-C (58), surfactant protein-A (59), telomerase reverse transcriptase (TERT), and telomerase RNA component (TERC) have been associated with familial interstitial pneumonia (FIP), a hereditary form of lung fibrosis (60). Genetically modified mouse models, based on known FIP mutations or common alleles found in IPF, offer valuable insights into the pathogenesis of this disease.

Silica Model

Inhalation of silica (quartz) dust is a known cause of lung fibrosis in humans, making silica administration a widely used method in animal models of the disease (61). Silica particles are believed to induce fibrosis through their uptake by macrophages, which then produce pro-fibrotic cytokines like TNF-α, platelet-derived growth factor (PDGF), and TGF-β. A major advantage of the silica model is the continuous stimulation provided by the slow clearance of silica particles from the lungs. However, the model also has limitations. The development of fibrotic nodules can take up to 16 weeks, and the resulting fibrosis lacks the characteristic histological features of usual interstitial pneumonia (UIP) seen in IPF. Moreover, the delivery of aerosolized silica requires specialized and expensive equipment. Similar to bleomycin-induced damage, the development of fibrosis in this model is species-dependent, with Balb/c mice exhibiting resistance to fibrosis development (62).

CONCLUSION

The animal experimental models have provided valuable insights into disease mechanisms and aided in identifying new therapeutic targets. However, it is important to note that no single model fully recapitulates human disease, and each has unique advantages and disadvantages. The use of experimental animals in modeling lung diseases presents certain limitations. While lung diseases in humans usually develop over a long period of time, this process can be accelerated in experimental animals. This may affect the natural course of the disease and response to treatment. In addition, ethical concerns and the welfare of experimental animals must also be taken into account. Awareness of these limitations is crucial for the accurate interpretation of research findings and their applicability to humans.

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