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Elastase-induced pulmonary emphysema: insights from experimental models

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ABSTRACT

Several distinct stimuli can be used to reproduce histological and functional features of human emphysema, a leading cause of disability and death. Since cigarette smoke is the main cause of emphysema in humans, experimental researches have attempted to reproduce this situation. However, this is an expensive and cumbersome method of emphysema induction, and simpler, more efficacious alternatives have been sought. Among these approaches, elastolytic enzymes have been widely used to reproduce some characteristics of human cigarette smoke-induced disease, such as: augmentation of airspaces, inflammatory cell influx into the lungs, and systemic inflammation. Nevertheless, the use of elastase-induced emphysema models is still controversial, since the disease pathways involved in elastase induction may differ from those occurring in smoke-induced emphysema. This indicates that the choice of an emphysema model may impact the results of new therapies or drugs being tested. The aim of this review is to compare the mechanisms of disease induction in smoke and elastase emphysema models, to describe the differences among various elastase models, and to establish the advantages and disadvantages of elastase-induced emphysema models. More studies are required to shed light on the mechanisms of elastase-induced emphysema.

Key words: chronic obstructive pulmonary disease, elastase, emphysema, experimental model, smoke.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a leading cause of death, progressive disability, and permanent impairment, imposing a huge economic and social burden worldwide (Pauwels and Rabe 2004). COPD is a term that refers to a large group of lung diseases characterized by the obstruction of air flow that interferes with normal breathing. Emphysema and chronic bronchitis are the most important conditions that compose COPD, and they present different physiopathology and symptoms. Emphysema has retained more attention since it is characterized by permanent inflammation and destruction of the alveolar walls, which leads to enlarged air spaces, loss of elastic recoil, reduced gas exchange capacity, and pulmonary hyperinflation. The most common risk factor for COPD is cigarette smoking. Other documented causes of COPD include occupational dusts and chemicals, as well as indoor air pollution from biomass cooking and heating in poorly ventilated dwellings. There are no efficient alternatives to treat this disorder. In contrast to the large amount of experimental research available for other pulmonary inflammatory diseases, experimental models of COPD have not appeared until recently (Lenssen and Stolk 2007, Takahashi et al. 2008, Hind and Stinchcombre 2009, Wilson et al. 2010). The limited availability of information regarding the mechanisms of COPD development and progress restricts the establishment of animal models. Nevertheless, some studies did provide insights on histological and functional aspects of human COPD (Sahebjami and Wirman 1981, Fournier and

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Lewis 2000, Kasahara et al. 2000, Taraseviciene-Stewart et al. 2005, De Paepe et al. 2008).

Different mechanisms seem to be involved in COPD pathophysiology: a) protease-antiprotease imbalance, leading to matrix destruction and emphysema, b) oxidative stress, promoting inflammatory cell influx, protein oxidation, and airway squamous metaplasia, c) alveolar matrix degradation and impaired regeneration ability in small airways and excessive matrix deposition in pulmonary arteries causing pulmonary hypertension, and d) apoptosis of endothelial and epithelial cells. Although smoking is undoubtedly the main cause of COPD, several approaches have been used to induce experimental emphysema. Among them, the protease-antiprotease hypothesis for emphysema development has attracted much attention, since it fits the scenario of inflammatory cell influx caused by the exposure to cigarette smoke with release of proteases that prevent the antiproteolytic response, resulting in matrix degradation and emphysema. However, several months and high frequency of smoke exposure are required to reproduce the characteristics of human emphysema in animal models. Besides, this approach also leads to acquired immunity with the stabilization of emphysema, and it is therefore time-consuming, expensive, and often ineffective. In order to bypass these limitations, other mechanisms have been proposed, mostly tissue-degrading approaches using elastolytic proteases (such as the porcine pancreatic elastase – *PPE*, human neutrophilic elastase, papain) and serine/cysteine proteases.

This review will focus on: 1) comparing the emphysema induction mechanisms of smoke and elastase models described in the literature, 2) describing the differences among various elastase experimental protocols, and 3) establishing the advantages and disadvantages of elastase-induced emphysema.

ELASTASE *VS.* SMOKE-INDUCED EMPHYSEMA

Elastase-induced emphysema is an interesting, low-cost approach (Table I), since a single administration may rapidly result in histological and morphological characteristics compatible with those of panacinar emphysema (Snider et al. 1986, Snider 1992). Conversely, prolonged smoke exposure is expensive, slow, and produces centrilobular emphysema (Wright and Churg 1990). Furthermore, the damage resulting from elastase emphysema is homogeneously distributed, while cigarette smoke particles remain in the bronchial tree until they are slowly delivered to the alveoli. Both models are important to study treatment strategies and, in humans, are correlated to genetic (alpha 1 antitrypsin deficiency) and smoke-induced emphysema, respectively (Table II).

It has been demonstrated that smoke emphysema models usually promote morphological changes compatible with mild emphysema independent of time of exposure (Rabe et al. 2007). In contrast, the severity of elastase-induced emphysema is related to enzyme dose. Since the majority of elastase protocols use a single intratracheal instillation, the extrapolation of results to the slowly developing features of human disease must be very careful because different mediators may be involved (Sawada et al. 2007, Rangasamy et al. 2009).

Loss of body and muscle weight in COPD (pulmonary cachexia) contributes to skeletal muscle weakness and impaired exercise capacity (Schols et al. 1998, Bolton et al. 2004). Pulmonary cachexia is associated with lung inflammation (Keatings et al. 1996) and increased levels of circulating inflammatory cytokines (Di Francia et al. 1994, Takabatake et al. 2000, Eid et al. 2001), suggesting that systemic inflammation could trigger or contribute to muscle atrophy (Langen et al. 2006). These and other systemic abnormalities can be reproduced with both smoke exposure and elastase challenge protocols (Gosker et al. 2009, Lüthje et al. 2009). Cigarette smoke induces weight loss, muscle atrophy, changes in muscle fiber type, and systemic inflammation, as well as reduces strength and endurance in experimental animals (Langen et al. 2006, De Paepe et al. 2008, Gosker et al. 2009), similarly to what occurs in humans. Few data are available regarding these aspects in elastase models (Lewis et al. 1992, Marchand et al. 2000, Degens et al. 2007, Lüthje et al. 2009).

In addition to inflammation, the development and progression of skeletal muscle dysfunction in COPD has been strongly associated with increased oxidative stress and production of reactive oxygen species (ROS), and/or with reduced antioxidant capacity. ROS can promote muscle proteolysis, inhibit muscle-specific protein expression, and increase muscle cell apoptosis (Barreiro et al. 2005). Cigarette smoke is an extremely concentrated source of ROS and reactive nitrogen species, and

TABLE I
Comparison between elastase *versus* smoke-induced emphysema features.

Features	Elastase emphysema	Smoke emphysema
Cost	Low	High
Duration	Brief	Long
Lesion type	Panacinar emphysema	Centrilobular emphysema
Emphysematous similarities	Genetic emphysema	Cigarette-induced emphysema
Severity	Depends on the enzyme dose	Mild/Moderate
Disease progression after stimuli cessation	Lesion maintenance	Stop progression/ Regression of lesions
Mediators involved	Unknown	Partially known
Systemic alterations presence	Yes	Yes
Oxidative stress presence	Yes	Yes
Apoptosis pathways	Caspase/TNF-TNFR	Caspase/Fas-FasL

TABLE II
Human and experimental emphysema induced by elastase and smoke.

Features	Human emphysema	Elastase-induced emphysema	Smoke-induced emphysema
Cachexia	Yes	Yes	Yes
Time of development	Long	Brief	Long
Progression of disease after stimuli cessation	Yes	No	No, regression of lesions
Endurance reduction	Yes	Yes	Yes
Epithelial and endothelial cell apoptosis	Yes	Yes	Yes
Extracellular matrix degradation	Yes	Yes	Yes
Type of lesion	Centrilobular/ Panacinar (genetic)/ Irregular	Panacinar	Centrilobular
Presence of oxidative stress	Yes	Yes	Yes
Respiratory muscle weakness	Yes	Yes	Yes
Severity	Mild/Moderate/ Severe	Depends on the enzyme dose	Mild
Presence of systemic alterations	Yes	Yes	Yes

the inflammatory response to smoke potentially augments oxidative stress (Hoidal et al. 1981, Ludwig and Hoidal 1982, Bridges et al. 1985, MacNee 2005a, b). Animal models also develop increased protein synthesis by lipid peroxidation products, such as 4-hydroxynonenal, and depletion of antioxidant substances, such as superoxide dismutase, catalase, ascorbic acid and glutathione (McCusker and Hoidal 1990, Churg and Cherukupalli 1993, Cavarra et al. 2001, Aoshiba et al. 2003a).

Few studies have investigated the association of protease-induced emphysema with oxidative stress (Mattson et al. 2002, Petrache et al. 2008, Borzone et

al. 2009). It has been evidenced that oxidant attack diminishes α1-antitrypsin (α1-AT) affinity for elastase by the oxidation of a methionine residue at its active site. As a result, higher amounts of elastase are available to degrade lung matrix components (Carp and Janoff 1979, Janoff et al. 1979, Carp and Janoff 1980, Clark et al. 1981, Matheson et al. 1981), worsening the elastase-induced protease/antiprotease imbalance (Fig. 1). Borzone and colleagues (2009) describe that the modulation of elastase over pulmonary glutathione metabolism depends on the animal species, suggesting a genetic-specific degree of oxidant-antioxidant imbalance (Borzone et al. 2009).

The conventional hypothesis for emphysema admits that cigarette smoke induces a huge influx of inflammatory cells into the lungs, which results in release of ROS and proteases, matrix degradation, and also death of structural cells. However, some studies have demonstrated that emphysema may develop due to cell apoptosis without an increased inflammation in mice (Kasahara et al. 2000, Aoshiba et al. 2003b, Petrache et al. 2005). In this line, recent data from animal (Sawada et al. 2007, Rangasamy et al. 2009) and human studies suggest an imbalance between apoptosis and the repair of structural cells in the lung, favoring the destruction of lung tissue in response to cigarette smoke, which would lead to emphysema. Smoke exposure interferes with cell proliferation, chemotaxis, and production/remodeling of matrix components by fibroblasts (Carnevali et al. 1998, Rennard et al. 2006), which partially explains the increased number of apoptotic cells in human lungs with severe emphysema (Yokohori et al. 2004, Imai et al. 2005). In COPD, apoptotic cells include alveolar and bronchial epithelial cells, as well as endothelial cells in the parenchyma. Apoptosis may persist even after smoking cessation.

Two main apoptotic pathways, extrinsic and intrinsic (mitochondrial), lead to DNA fragmentation and cell death. The extrinsic pathway is triggered mostly by death ligands, such as tumor necrosis factor (TNF), Fas ligand (FasL), and TNF-related apoptosis-inducing ligand (TRAIL) through their respective receptors. Following receptor activation, intracellular death domains autophosphorylate, and caspases, such as caspase-8/10 (cystein proteases), are activated, cleaving specific substrates and activating other caspases. These cleaved substrates will transmit the apoptotic signal to the nucleus or mitochondria (caspase-3, 6, 7), or interfere with anti-apoptotic protection, or activate the intrinsic pathway. The intrinsic pathway, also known as mitochondrial pathway, is mainly triggered by cellular stresses that cause DNA damage, such as oxidative stress. In the mitochondrial pathway, permeability of the mitochondrial membrane increases, allowing the release of cytosilic cytochrome *c*. Cytochrome *c* will join Apaf-1 and caspase-9 to form the so-called apoptosome (activated caspase-9) that will activate caspase-3. Increased apoptosis in the human emphysematous lung has been frequently reported (Yokohori et al. 2004, Imai et al. 2005). In smoke-exposed mice lungs, an increase in apoptotic cells has also been described, and may be associated not only with the extrinsic pathway, with enhanced expression of FasL and caspase, but also with mitochondrial pathway activation, with increased expression of cytochrome *c* oxidase (Kang et al. 2006, Rangasamy et al. 2009) – suggesting that, in experimental smoke-induced emphysema, apoptosis is mediated by both pathways.

Using a FasL deficient (lpr) mouse model, Sawada and colleagues (2007) demonstrated that apoptosis in elastase-induced emphysema was not mediated by Fas/FasL interaction (Sawada et al. 2007). However, the extrinsic pathway can also be activated by other members of the TNF superfamily, including TNF receptor, TRAIL-1/TRAIL-2, and lymphotoxin β receptor. Since apoptosis was attenuated in a TNF-α receptor-deficient mouse model of elastase-induced emphysema (Lucey et al. 2002), it could be speculated that apoptosis was mediated via TNFR/TNF interaction in that model. A brief schematic representation of the mechanisms of action of elastase related to the activation of the intrinsic apoptosis pathway is shown in Figure 2. Together, these data support the idea that the pathophysiology of smoke-induced emphysema differs from that of elastase emphysema. The pathophysiological specificities of each model still require further investigation.

COMPARISON OF ELASTASE MODELS

A single dose of elastase leads to airspace enlargement inducing emphysema-like lesions (Lucey et al. 1998, Kononov et al. 2001, Inoue et al. 2003, Ishizawa et al. 2004, Ito et al. 2005, Kawakami et al. 2008). In gen-

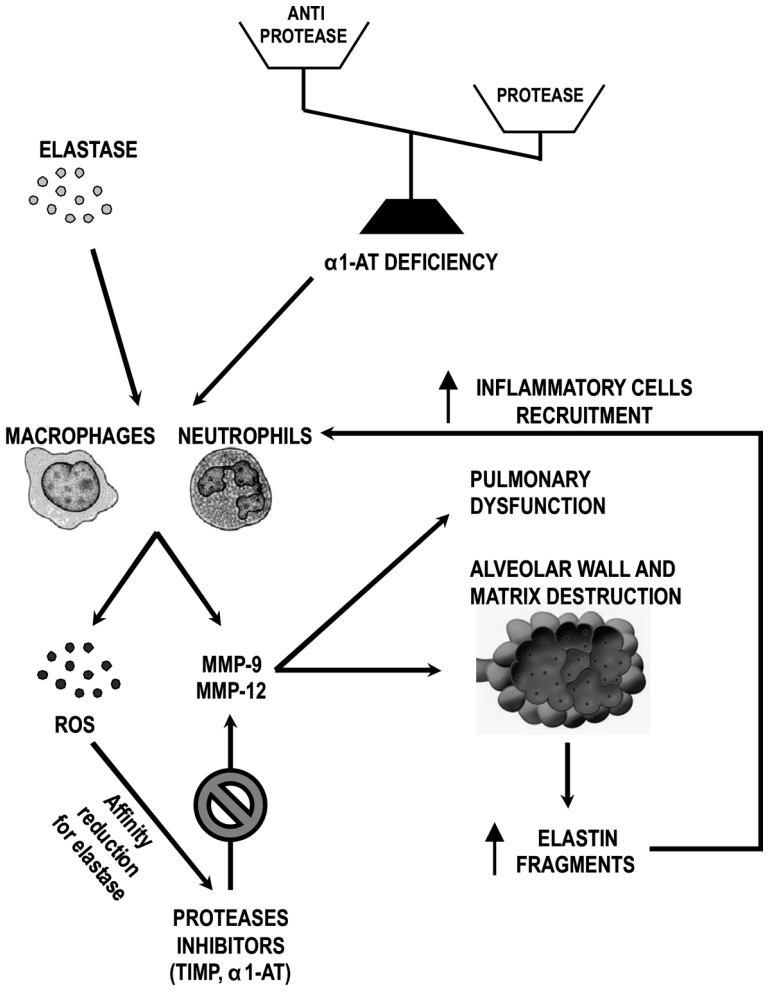

Fig. 1 – Elastase action in the lung. Either elastase instillation or genetic deficiency of α1-antitrypsin (α1-AT) induces a proteolytic-antiproteolytic imbalance, favoring a proteolytic bias. Elastolytic enzymes may augment inflammatory cell influx into the airspaces, which in turn promotes release of matrix metalloproteases (MMP) and reactive oxygen species (ROS). The matrix-degrading capacity of MMP causes destruction of alveolar septa, increases airspaces, and causes pulmonary and cardiovascular dysfunction (such as air trapping, hypercapnia, pulmonary arterial hypertension, and right ventricle hypertrophy). Elastin fragments resulting from alveolar destruction become chemoattractants to further inflammatory cell influx.

eral, elastolytic injury progresses to destructive effects induced by host proteases produced and activated by inflammatory cells, mainly neutrophils. Several elastase emphysema protocols are able to develop pulmonary function abnormalities, such as hypoxemia and secretory cell metaplasia (Breuer et al. 1993, Kononov et al. 2001, Ito et al. 2005, Lüthje et al. 2009).

Most systemic manifestations of emphysema have been reproduced in mice using repeated elastase challenges, and persisted during prolonged periods after the lesion is induced (Lüthje et al. 2009). Kawakami and colleagues (2008) held a sequential and quantitative analysis using micro-computed tomography in a murine model of elastase emphysema, demonstrating the persistence of morphological changes up to four weeks after elastase instillation (Kawakami et al. 2008). Other investigators have observed persistent elastase emphysema up to eight weeks and more after elastase instillation (Otto-Verbene et al. 1992). Diaphragm shortening and weakness, skeletal muscle impairment, muscle fiber pattern and exercise intolerance, which are among the cellular and biochemical adaptations that characterize severe human COPD, have been reproduced in elastase emphysema (Supinski and Kelsen 1982, Fournier

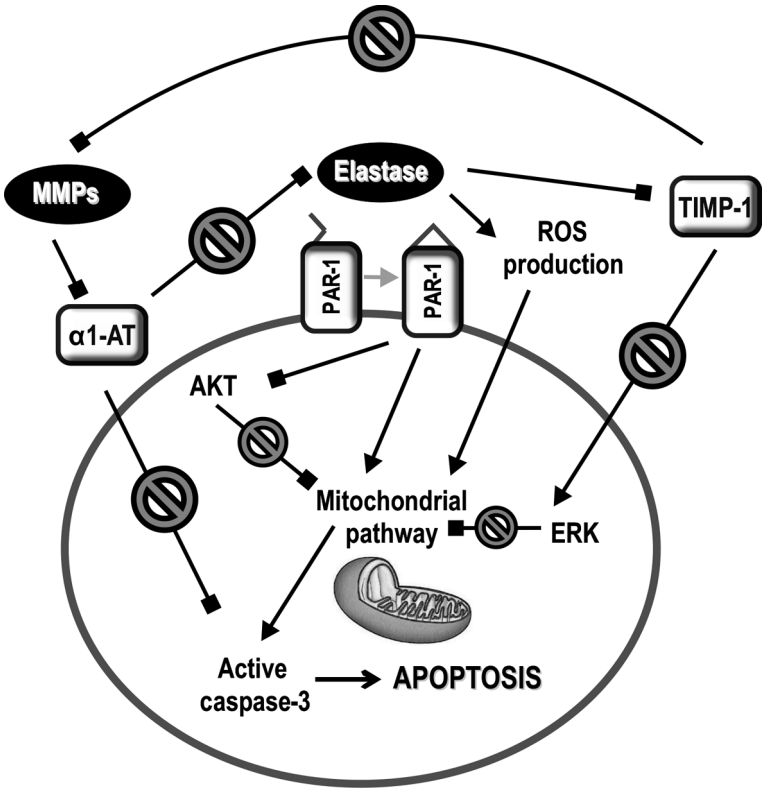

Fig. 2 – Activation of the intrinsic pathway of apoptosis in elastase emphysema. Elastase, mainly released by activated neutrophils and macrophages, may induce small airway and alveolar epithelial cell apoptosis through the intrinsic (mitochondrial) pathway and by decreasing a serine/threonine protein kinase phosphorylation (AKT, anti-apoptotic factor), following proteinase-activated receptor (PAR)-1 activation. Mitochondrial apoptosis pathway can also be activated by reactive oxygen species (ROS) production induced by elastase instilled in the lung. Protease inhibitor α1-antitrypsin (α1-AT) and tissues inhibitor of metalloproteinases (TIMP)-1 might act as survival factors by inhibiting active caspase, and through extracellular signal-regulated kinase (ERK) and AKT activation, respectively. Due to the bias favoring proteolytic production, α1-AT and TIMP-1 fail to prevent lung endothelial cell death in emphysema.

and Lewis 2000, Mattson et al. 2002, Degens et al. 2007, Lüthje et al. 2009). Lüthje and colleagues (2009) have observed that repeated instillations of elastase induce a more severe emphysematous state than single dose. The main changes observed were decreased exercise capacity, body weight loss, pulmonary hypertension, and urinary norepinephrine concentration. Besides, with multiple doses, increased right ventricular mass and diaphragmatic dysfunction were perpetuated for up to six months (Lüthje et al. 2009).

The protease/anti-protease imbalance hypothesis of pulmonary emphysema was based on observations that smokers with α1-antitrypsin deficiency were at increased risk to develop emphysema. Since then, a variety of enzymes with the ability to degrade intact elastin have been instilled into the lungs of animals to produce emphysema (increase in airway resistance and decrease in pulmonary elastance), such as plant protease (papain), human neutrophil elastase (HNE) or porcine pancreatic elastase (PPE). In murines, the most consistent and impressive airspace enlargement has been accomplished by the intratracheal instillation of PPE. In studies employing papain, elastase activity depended on the purity and source of papain (Lieberman 1976). The quality of commercially available HNE for experimental use is consistent, but the cost of HNE is still high. PPE is cheap and easy to obtain, which makes it the most used protease. PPE and HNE have distinct primary endogenous inhibitors: α2-macroglobulin for PPE (α1-antitrypsin also may be effective) and α1-antitrypsin for

HNE (Stone et al. 1988), as well as distinct exogenous inhibitors, which may selectively inhibit these two proteases (Lai and Diamond 1990, Lafuma et al. 1991).

Interspecies differences in genetic background interfere with the severity of elastase emphysema development. Compared to mice and rats, hamsters are easily susceptible to lung inflammation, hemorrhage, and oxidative stress (Corteling et al. 2002, Borzone et al. 2009). Therefore, hamsters are often used in experimental COPD models. It has been reported that hamsters present relatively low levels of α1-antitrypsin, which would favor emphysema caused by proteolytic attack on the lung (Lieberman 1976, Jannof 1985). Borzone and colleagues (2009) have used one single protocol (fixed dose/100 g body weight) to demonstrate different interspecies response (Borzone et al. 2009). They evidenced that hamster lungs are highly susceptible to injury by elastase and present early total glutathione depletion and significant inhibition of the main enzymes involved in glutathione metabolism (γ-glutamylcysteine, glutathione peroxidase, glutathione reductase). In turn, rat lungs are less susceptible to elastase injury, and exhibit subtle or no reduction in glutathione content or in glutathione-related enzyme activities after elastase instillation (Borzone et al. 2009). The induction of emphysema is thus easier in hamsters because of the low levels of α1-antitrypsin in this species.

Recently, emphysema progression has been attributed not only to elastin degradation, but also to abnormal repair of lung tissue (remodeling of collagen), culminating in small airway fibrosis and pulmonary dysfunction. Several elastase-induced emphysema models have been used to investigate the mechanisms involved in this remodeling process (Lucey et al. 1998, Kononov et al. 2001, Ito et al. 2005, Rubio et al. 2004, Hoffman et al. 2010). Rubio and colleagues (2004) demonstrated the therapeutic effects of a glutathione precursor (N-acetylcysteine) at attenuating collagen deposition in rat lungs using a single elastase instillation protocol (Rubio et al. 2004). Kononov and colleagues (2001) showed the deformation of the elastin-collagen network after a single dose of PPE in rats. They evidenced that newly deposited elastin and collagen fibers undergo significantly larger distortions during stretching than normal tissue. There is also a reduction in the threshold for mechanical failure of collagen, conferring mechanical instability to pulmonary tissue (Kononov et al. 2001). Hoffman and colleagues (2010) demonstrated that extracellular matrix quality influences the regenerative capacity of the lung and the patterns of cell proliferation in lungs of adult mice. Also, these authors observed that, as in human disease, elastase injury leads to a reduction of baseline progenitor cells in mice lung (Hoffman et al. 2010).

ADVANTAGES/DISADVANTAGES OF ELASTASE EMPHYSEMA MODEL

Elastase protocols are quick and inexpensive. Lesion severity is modulated by enzyme dose, and the induced morphological and functional changes are detectable in the long term. Therefore, elastase emphysema has become a useful tool to validate a variety of new drugs and interventions, such as simvastatin (Takahashi et al. 2008) and bone marrow-derived cell therapy (Ishizawa et al. 2004), or to develop mechanistic investigations of severe pulmonary tissue abnormalities (Sawada et al. 2007, Kawakami et al. 2008). Additionally, several features of human emphysema (genetic or cigarette smoke-induced) have been reproduced by elastase emphysema, including systemic inflammation and chronic adaptations (Table II).

Although elastase also causes most of the features observed in cigarette smoke-induced emphysema, the differences in injury pathway remain to be clarified, and the onset and duration of lesions differ in both models.

CONCLUSION

COPD is a complex disorder. Even though smoking is the gold standard for experimental emphysema models, its limitations have led scientists to seek new approaches in order to simulate the morphologic and functional alterations of human disease. Different studies have been developed using elastolytic enzymes in distinct doses and times of administration, but the ideal protocol is still unknown. Emphysema models induced by elastase present some advantages, such as prolonged histological changes and reduction in the cost of emphysema research. However, more studies are required to shed light on the mechanisms of elastase-induced emphysema, so that new therapeutic strategies can be developed.

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RESUMO

Diversos estímulos podem ser utilizados para reproduzir características histológicas e funcionais do enfisema humano, uma das principais causas de incapacidade e morte. Uma vez que a fumaça de cigarro é a principal causa de enfisema em humanos, estudos experimentais têm tentado reproduzir esta situação. No entanto, esse é um método dispendioso e complicado para a indução do enfisema e, alternativas mais simples e eficazes, têm sido pesquisadas. Entre essas abordagens, enzimas elastolíticas vêm sendo amplamente utilizadas para reproduzir algumas das características do enfisema humano, tais como: aumento dos espaços aéreos, influxo de células inflamatórias nos pulmões e inflamação sistêmica. Entretanto, o uso de modelos de enfisema induzido por elastase permanece controverso, uma vez que as vias de ação da doença envolvidas na indução com elastase podem diferir das que ocorrem no enfisema induzido pelo fumo. Isso indica que a escolha de um modelo de enfisema pode influenciar os resultados de novas terapias ou drogas a serem testadas. O objetivo desta revisão é comparar os mecanismos da indução da doença em modelos de enfisema por fumaça e elastase, descrever as diferenças entre os vários modelos de elastase e, estabelecer as vantagens e desvantagens dos modelos de enfisema por elastase. Mais estudos são necessários para elucidar os mecanismos relacionados ao enfisema induzido por elastase.

Palavras-chave: doença pulmonar obstrutiva crônica, elastase, enfisema, modelo experimental, fumaça.

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