Rev Port Pneumol. 2012;18(3):128–136

revista portuguesa de
PNEUMOLOGIA
portuguese journal of pulmonology
www.revportpneumol.org

ORIGINAL ARTICLE

Alterations in pulmonary structure by elastase administration in a model of emphysema in mice is associated with functional disturbances

D. Vidal[a,b], G. Fortunato[a,c], W. Klein[d,e,f], L. Cortizo[a], J. Vasconcelos[a,b], R. Ribeiro-dos-Santos[a,b], M. Soares[a,b], S. Macambira[a,b,g,*]

[a] *Laboratório de Engenharia tecidual e Imunofarmacologia, Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, BA, Brazil*
[b] *Centro de Biotecnologia e Terapia Celular, Hospital São Rafael, Salvador, BA, Brazil*
[c] *Programa de Pós-Graduação em Biotecnologia, Universidade Estadual de Feira de Santana, Feira de Santana, BA, Brazil*
[d] *Faculdade de Biologia, Instituto de Biologia, Universidade Federal da Bahia, Salvador, BA, Brazil*
[e] *Instituto de Biociências, Instituto Nacional de Ciência e Tecnologia em Fisiologia Comparada, UNESP, Rio Claro, SP, Brazil*
[f] *Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, SP, Brazil*
[g] *Laboratório de Eletrofisiologia Cardíaca ''Prof Antônio Paes de Carvalho'', Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, RJ, Brazil*

Received 6 July 2011; accepted 23 December 2011
Available online 17 March 2012

KEYWORDS
Pulmonary emphysema;
Ergometric test;
Porcine elastase;
C57Bl/6

Abstract Several experimental studies of pulmonary emphysema using animal models have been described in the literature. However, only a few of these studies have focused on the assessment of ergometric function as a non-invasive technique to validate the methodology used for induction of experimental emphysema. Additionally, functional assessments of emphysema are rarely correlated with morphological pulmonary abnormalities caused by induced emphysema. The present study aimed to evaluate the effects of elastase administered by tracheal puncture on pulmonary parenchyma and their corresponding functional impairment. This was evaluated by measuring exercise capacity in C57Bl/6 mice in order to establish a reproducible and safe methodology of inducing experimental emphysema. Thirty six mice underwent ergometric tests before and 28 days after elastase administration. Pancreatic porcine elastase solution was administered by tracheal puncture, which resulted in a significantly decreased exercise capacity, shown by a shorter distance run (−30.5%) and a lower mean velocity (−15%), as well as in failure to increase the elimination of carbon dioxide. The mean linear intercept increased significantly by 50% in tracheal elastase administration. In conclusion, application of elastase by tracheal function in C57Bl/6 induces emphysema, as validated by

* Corresponding author.
E-mail address: simone@bahia.fiocruz.br (S. Macambira).

0873-2159/$ – see front matter
doi:10.1016/j.rppneu.2011.12.007

morphometric analyses, and resulted in a significantly lower exercise capacity, while resulting in a low mortality rate.

PALAVRAS-CHAVE
Enfisema pulmonar;
ensaio ergométrico;
Elastase suína;
C57Bl/6

Alterações na estrutura pulmonar devido à administração de elastase num modelo de enfisema em ratos encontram-se associadas a distúrbios funcionais

Resumo Vários estudos experimentais de enfisema pulmonar em modelos animais têm sido descritos na literatura científica. No entanto, apenas alguns destes estudos têm sido concentrados na avaliação da função ergométrica como técnica não-invasiva para validar a metodologia utilizada para a indução do enfisema experimental. Além disso, as avaliações funcionais de enfisema raramente se encontram correlacionadas com anomalias morfológicas pulmonares causadas por enfisema induzido. O presente estudo teve como objetivo avaliar os efeitos da elastase administrada por punção traqueal no parênquima pulmonar e a sua disfunção funcional correspondente. Esta foi avaliada através da medição da capacidade de exercício em ratos C57Bl/6, de forma a estabelecer uma metodologia reprodutível e segura de induzir o enfisema experimental. Trinta e seis ratos foram submetidos a testes ergométricos antes e 28 dias após a administração de elastase. A solução de elastase pancreática suína foi administrada por punção traqueal, o que resultou numa diminuição significativamente da capacidade de exercício, demonstrada pela diminuição da distância percorrida (menos de 30,5%) e por uma velocidade média inferior (menos de 15%), assim como pela incapacidade de aumentar a eliminação de dióxido de carbono. A intersecção linear média aumentou significativamente em 50% na administração traqueal da elastase. Em conclusão, a aplicação de elastase por punção traqueal em ratos C57Bl/6 induz enfisema, conforme foi validado por análises morfométricas, e resultou numa capacidade de exercício significativamente mais baixa, embora se tenha obtido uma baixa taxa de mortalidade.

Introduction

Emphysema is a chronic obstructive pulmonary disease (COPD), limiting air flow during expiration, and is associated with an abnormal inflammatory response of the lungs to noxious particles or gases (mainly cigarette smoke). This disease is characterized by a permanent abnormal dilatation of alveolar spaces. The destruction of pulmonary parenchyma impairs alveolar gas exchange, compromising the physical capacity of a patient since it is associated with airflow limitations that are not fully reversible.

No therapy capable of reversing emphysematous tissue lesions is available to date and, in severe chronic stages the only treatment that remains is lung transplantation, representing a procedure with high levels of morbidity and mortality. Emphysema, together with other types of COPD, are responsible for more than 2.5 million deaths every year, representing the fifth leading cause of mortality in the world.

The severity of its pathology together with the lack of any effective treatment transforms emphysema into a great medical challenge. There is a need for studies aiming to understand the pathogenic cellular mechanisms causing tissue destruction in order to elucidate the disease and to open new therapeutic avenues. Another aggravator is the fact that a considerable variation exists in the course of the disease among different smokers. Only about 15% of smokers develop COPD.[1,2]

Animal models represent a fundamental instrument to correlate pre-clinical research with clinical studies. The experimental model allows the detailed investigation of different factors influencing emphysema. These factors include inflammatory cell recruitment, genetic background, abnormal matrix repair, lung cell apoptosis, and misbalance between apoptosis and replenishment of structural cells in the lung. Additional factors are research of potential therapeutic agents and strategies, such as administration of stem cells or different growth factors.[3]

Until now there are many experimental models of COPD, each of them with advantages and disadvantages. In reality, a number of approaches have been tried because until now none of them constitutes a model that exactly reproduces all phases of development and clinical features of emphysema or any other abnormalities that make up this clinical entity, the COPD. Other factor that affects the reproducibility of data is the differences in anatomical structure with respect to development, maturation, structural organization of respiratory branches, and constituent cells and vascularization.[4] On the other side, some common characteristics can be observed between animal model and human disease. For example, rodents develop the phenomenon of metaplasia induced by reaction to injury,[5] which is a frequently observed response to an insult in different organs in humans, such as in respiratory tract, urinary bladder, and esophagus.

The first animal model of emphysema was developed more than 40 years ago[6] and it was evoked in rats by intratracheal instillation of the plant proteinase papain. The experimental models of emphysema are continuously improving and became a research tool for