ORIGINAL ARTICLES

Markers of Airway Remodeling in Induced Sputum From Healthy Smokers

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OBJECTIVE: Airway remodeling in chronic obstructive pulmonary disease (COPD) has been linked to the equilibrium between matrix metalloproteinase (MMP) 9 and its inhibitor, tissue inhibitor of metalloproteinase (TIMP) 1. However, that equilibrium has not been analyzed in healthy smokers. The aim of this study was to assess the equilibrium between MMP-9 and TIMP-1 in induced sputum from healthy smokers, healthy nonsmokers (controls), and patients with COPD.

PATIENTS AND METHODS: Samples of induced sputum were obtained from 35 individuals: 12 healthy smokers, 12 controls, and 11 patients with COPD. In each sample, a differential cell count was performed and enzyme-linked immunosorbent assays were used to analyze the concentrations of MMP-9 (total and active fraction) and TIMP-1.

RESULTS: Compared with controls, healthy smokers were found to have a higher mean (SD) concentration of total MMP-9 (273 [277] ng/mL vs 128 [146] ng/mL) and a higher ratio of total MMP-9 to TIMP-1 (0.16 [0.14] vs 0.08 [0.06]). However, the ratio of active MMP-9 to TIMP-1 was similar in the 2 groups. Samples from patients with COPD had the highest concentrations of total MMP-9 (477 [262] ng/mL) and active MMP-9 (178 [126] ng/mL) and the lowest concentrations of TIMP-1 (1.044 [1.036] µg/mL). When all groups were considered together, there was an inverse relationship between the MMP-9/TIMP-1 ratio and the forced expiratory volume in the first second (FEV_1). The relationship between the active MMP-9/TIMP-1 ratio and FEV_1 was even stronger, and the relation of both ratios with FEV_1 became stronger still when smoking was considered.

CONCLUSIONS: Healthy smokers had a higher concentration of total MMP-9 and that concentration was correlated with their exposure to tobacco smoke. Maintenance of the active MMP-9/TIMP-1 ratio in healthy smokers may explain the absence of progressive airway obstruction. Measurement of active MMP-9 concentration could be useful for assessment of airway remodeling.

Key words: *Matrix metalloproteinase 9. Airway remodeling. Healthy smokers.*

Marcadores de remodelado bronquial en el esputo inducido de fumadores sanos

OBJETIVO: El remodelado bronquial en la enfermedad pulmonar obstructiva crónica (EPOC) se ha relacionado con el equilibrio entre la metaloproitenasa (MMP) 9 y su inhibidor, el inhibidor tisular de MMP tipo 1 (TIMP-1). Dicho equilibrio no se ha analizado en fumadores sanos. Nuestro objetivo ha sido estudiar dicho equilibrio en el esputo inducido de fumadores sanos respecto a sanos no fumadores (controles) y pacientes con EPOC.

PACIENTES Y MÉTODOS: Se obtuvieron 35 muestras de esputo inducido, de las que 12 provenían de fumadores sanos, otras 12 de controles y 11 de pacientes con EPOC. Se estudiaron la celularidad de las muestras y la concentración de MMP-9 (total y fracción activa) y TIMP-1 mediante enzimoinmunoanálisis.

RESULTADOS: Los fumadores sanos mostraron mayor concentración media (± desviación estándar) de MMP-9 total (273 ± 277 ng/ml) y una ratio mayor (0,16 ± 0,14) que los controles (128 ± 146 ng/ml y 0,08 ± 0,06, respectivamente). Sin embargo, la ratio MMP-9 activa/TIMP-1 fue equiparable en ambos grupos. Los pacientes con EPOC mostraron los valores más altos de MMP-9 total (477 ± 262 ng/ml) y activa (178 ± 126 ng/ml) y los más bajos de TIMP-1 (1.044 ± 1.036 ng/ml). Globalmente, la ratio mostró una relación inversa con el volumen espiratorio forzado en el primer segundo. Dicha relación fue aún superior con la MMP-9 activa y con el grado de tabaquismo.

CONCLUSIONES: Los fumadores sanos presentaron una mayor concentración de MMP-9 total en relación con el grado de exposición tabáquica. Una ratio MMP-9 activa/TIMP-1 conservada en fumadores sanos podría explicar la ausencia de obstrucción progresiva de la vía aérea. La medida de la MMP-9 activa puede ser útil en la determinación del remodelado bronquial.

Palabras clave: *Metaloproteinasa 9 (MMP-9). Remodelado bronquial. Fumadores sanos.*

Introduction

Mortality due to smoking-related respiratory disease, not including lung cancer, has increased in recent years to become a significant health problem.[1] However, the effects and time course of airway pathology caused by exposure to tobacco smoke are still not known with any

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certainty. Various studies have demonstrated that exposure to tobacco smoke causes cellular oxidative stress and the release of inflammatory mediators in the airways of healthy subjects, and that these effects can be both acute and chronic.[2,3] In susceptible individuals, these processes would lead to structural changes in the small airway that would cause progressive loss of lung function. The appearance of chronic progressive airflow limitation in part reflects the lung remodeling that characterizes chronic obstructive pulmonary disease (COPD).[4] Some enzymes, such as matrix metalloproteinase (MMP) 9, have been linked to COPD, in which an increasing number of studies have described a local overproduction of MMP-9 and an altered ratio between this enzyme and its inhibitor, tissue inhibitor of metalloproteinase 1 (TIMP-1).[5,6] The usefulness of this enzyme as a marker of remodeling has also been described.[7,8]

MMPs—in particular MMP-1, MMP-2, and MMP-9—are collagenases that regulate the homeostasis of the lung matrix, which is made up principally of collagen and other proteins.[9,10] The direct effect of tobacco smoke on these enzymes is poorly understood. Finlay et al[11] were the first to describe an increase in the levels of MMP-9 associated with COPD, finding elevated levels of the enzyme in bronchoalveolar lavage fluid from a subgroup of individuals within a small sample of smokers. More recently, Kang et al[12] described increased expression of MMP-9 seen in bronchial biopsies from smokers compared with nonsmokers; that increase was correlated with the number of cigarettes smoked. It has recently been suggested that the equilibrium between these proteases and their inhibitors would better reflect or display a stronger correlation with the intensity of the disease.[7] Very little data is available on TIMP-1 in smokers with which to assess the effect of smoking on structural changes.

The aim of this study was to analyze the ratio between MMP-9 and TIMP-1 in samples of induced sputum from healthy smokers and healthy nonsmokers. A third group of subjects with pulmonary disease caused by smoking (COPD group) was included as a reference. In addition, the fraction of active MMP-9 was analyzed and compared with results provided by analysis of the total concentration of the enzyme.

Patients and Methods

Selection of Patients

A total of 37 volunteers were recruited: 26 healthy subjects, of whom 13 were smokers and 13 nonsmokers, and 11 patients with COPD. Healthy nonsmokers were mostly volunteers from among hospital staff. Healthy smokers were recruited from subjects who had registered with the smoking cessation clinic but had yet to begin the cessation program. Patients with COPD were consecutively recruited in the outpatient pneumology department. All patients gave signed informed consent for inclusion in the study, which was approved by the hospital's ethics committee. Volunteers were considered to be healthy nonsmokers if they had no prior history of significant respiratory disease, their results for forced spirometry and bronchodilator test were within the limits of reference values from the Spanish Society of Pulmonology and Thoracic Surgery (SEPAR),[13] and they had never smoked. Volunteers were considered healthy smokers if they met all of the above criteria, except the last, and were also active smokers.

Criteria for inclusion in the COPD group were as follows: chronic airflow limitation according to the definition of the Global Initiative for Chronic Obstructive Lung Disease[1] and a negative bronchodilator test (<12%) in the stable phase (recorded in the patient's chart). All recruited patients continued with their normal treatment, which had not changed for at least 1 month prior to the study. Exclusion criteria for patients with COPD were exacerbation of the disease or the presence of extensive infiltrates, bronchiectasis, or pleural inflammation and thickening in chest radiographs, severe alcoholism, cancer, inability to cooperate, or severe heart, liver, or kidney failure. Table 1 shows the clinical characteristics and lung function for all groups.

Study Design and Methods

A cross-sectional, observational study was designed to compare the 3 groups of subjects: healthy nonsmokers (control group), healthy smokers, and patients with COPD. In all subjects a history was taken and a physical examination performed, and sputum was induced according to the method described below. Samples were sent to the cytology laboratory for immediate processing. The supernatant was frozen at –80ºC and sent to the biochemistry laboratory for processing.

Sputum induction. Sputum was induced in healthy subjects according to a slightly modified version of the technique described by Belda et al.[14,15] A 3% saline solution was nebulized for 7 minutes, 10 minutes after administration of salbutamol (200 µg) through a spacer chamber. If sputum was not obtained, the concentration of the saline was progressively increased (4%, 5%, etc) every 7 minutes. In the case of patients with stable COPD, isotonic saline was used initially. If it was well tolerated and a valid sputum sample was not obtained, nebulization was continued for 7 minutes with hypertonic saline at a concentration of 3%, and this was repeated again at the same concentration if no result was obtained. Droplets with a mean diameter of 7 µm were produced at a rate of 3 mL/s using an ultrasound nebulizer (Omron NE U07, Omron Healthcare Europe, Hoofddorp, Holland). Spirometry was performed according to SEPAR guidelines[13] as a safety measure at the beginning of each inhalation period and at the end of sputum induction. The patient was then instructed in the best maneuvers to achieve effective expectoration. All patients rinsed their mouth and blew their nose to limit, as far as possible, contamination of the induced sputum, which was collected in a sterile container.

Sputum processing. Within a maximum of 2 hours, mucus plugs were separated from saliva in the expectorated samples. Mucus plugs were homogenized in a volume of fresh 0.1% dithiothreitol equivalent to 4 times the weight of the plugs. Later, an equal volume of phosphate buffered saline was added and the solution was filtered through a 41 µm nylon mesh. A 10 µL aliquot of this solution was taken for analysis of cell viability and the remainder was centrifuged for 10