CHEST

Original Research

COPD

Measurements of Desmosine and Isodesmosine by Mass Spectrometry in COPD*

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Objectives: **Application of mass spectrometry (MS) for direct measurements of desmosine (D) and isodesmosine (I) in urine, plasma, and sputum as markers of elastin degradation in patients with α_1-antitrypsin deficiency (AATD) and non-AATD-related COPD.**

Background: **In COPD patients, the lungs undergo elastin injury, which can be monitored by measurements of D and I in body fluids as specific markers of elastin degradation using the specificity and sensitivity of MS.**

Methods: **Acid hydrolysis of blood plasma, 24-h urine and sputum measurements, followed by chromatographic separation for mass spectrometric analysis.**

Results: **Each patient group had levels of plasma D and I that were statistically significantly higher than those of control subjects. AATD patients had higher levels than COPD patients with normal α_1-antitrypsin (AAT) levels. Twenty-four-hour urine measurements demonstrated no significant difference in total levels of D and I among control subjects and patients but showed a free (unbound) concentration of D and I in urine, which was statistically significantly higher in patients with COPD with and without AAT. The D and I levels in the sputum of patients with AATD exceeded the levels in COPD patients with normal AAT levels.**

Conclusions: **MS allows a sensitive and specific analysis of D and I in body fluids. The quantification of D and I in sputum, along with increases of D and I in plasma and an elevated free component of D and I in urine provide indexes that characterize patients with COPD and can be followed in relation to the course of the disease and/or therapy.**

(CHEST 2007; 131:1363–1371)

Key words: α_1-antitrypsin deficiency; COPD; desmosine; elastin; emphysema; isodesmosine; liquid chromatography; mass spectrometry

Abbreviations: AAT = α_1-antitrypsin; AATD = α_1-antitrypsin deficiency; D = desmosine; I = isodesmosine; HPLC = high-performance liquid chromatography; LC = liquid chromatography; MS = mass spectrometry

Lung elastin degradation occurs with the development of pulmonary emphysema in patients with COPD related to smoking or related to α_1-antitrypsin deficiency (AATD).[1,2]

Desmosine (D) and isodesmosine (I), the cross-linking amino acids that are present only in elastin in human beings, offer the prospect of assessing elastin degradation in patients with disease by measuring them in certain body fluids.[3] Thus far, D and I have been measured in the urine of

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This work was supported by funds from the James P. Mara Center for Lung Disease, the Flight Attendants Medical Research Institute, the Charles A. Mastronardi Foundation, the Ned Doyle Foundation, and the Alpha One Foundation, and by funds from Ethel Kennedy, John Kennedy, and Judith Sulzberger.

The authors have reported to the ACCP that no significant conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.

Manuscript received October 2, 2006; revision accepted December 26, 2006.

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DOI: 10.1378/chest.06-2251

patients with COPD and have been found to be statistically significantly elevated compared to healthy control subjects.[4–7] One study[8] demonstrated the daily variability of the excretion of D and I and did not show a statistically significantly elevated excretion of these amino acids in patients in 24-h collections. In this same study, statistically significantly increased excretion of D and I was found in patients with cystic fibrosis.

Peptides of elastin have been measured in plasma by radioimmunoassay and were found to be elevated in patients with COPD.[7,9] Because of the variability of the specificity of antibodies to elastin peptides, the quantitation of peptides has varied among various studies.[10] Direct measurements of D and I in plasma have not been recorded in healthy subjects or patients with COPD, and measurements of D and I in sputum have only been reported in 2003.[11] We now report measurements of D and I in plasma as well as in urine and sputum. The results demonstrate a statistically significant difference between healthy control subjects and patients in whom COPD has been diagnosed, and further suggest that measurements of D and I in plasma may be a discriminating index distinguishing patients with COPD from healthy subjects. D and I were measured in plasma, urine, and sputum in a cohort of patients in whom COPD related to smoking had been diagnosed and in a second cohort of patients in whom COPD was related to Z-phenotype AATD as well as smoking.

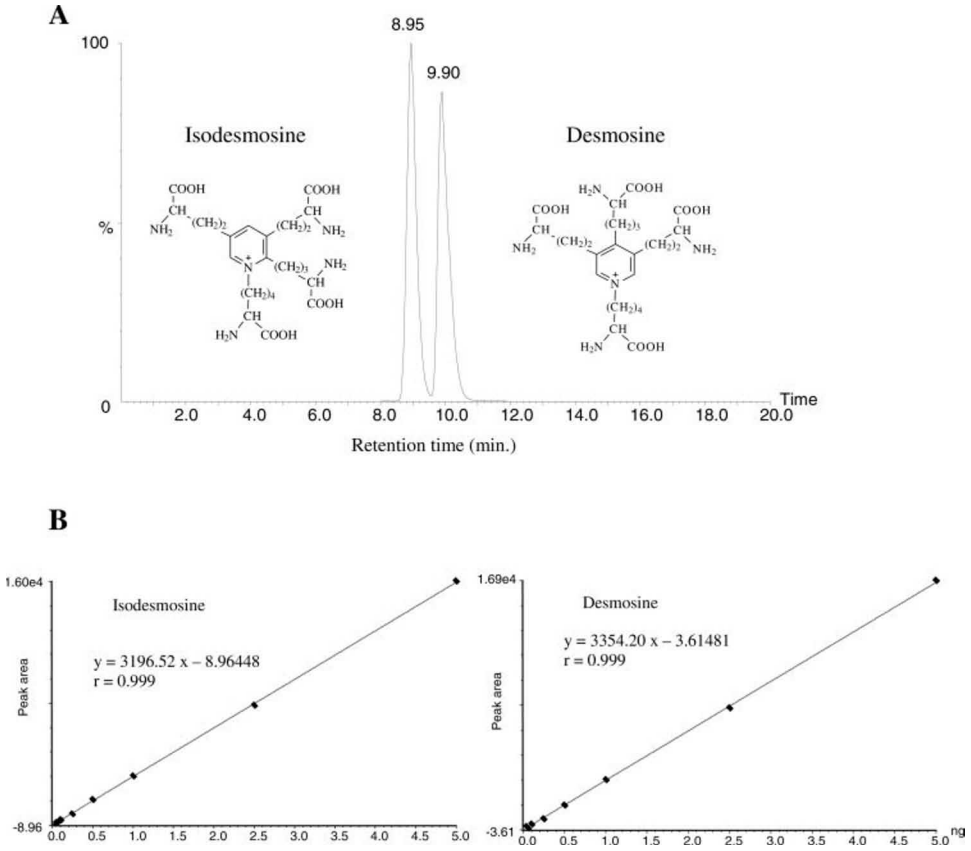

FIGURE 1. *Top*, *A*: the LC/MS ionchromatogram. HPLC separation of D and I was achieved by an Atlantis dC18 column (2.1 × 150 mm, 3 μm) [Waters]. The mobile phase A is aqueous 7 mmol/L heptafluorobutyric acid and 5 mmol/L ammonium acetate, and the mobile phase B is a solution of 7 mmol/L heptafluorobutyric acid and 5 mmol/L ammonium acetate in a acetonitrile/water (8:2 ratio). HPLC was performed using a 12-min linear gradient flow of the mobile phase A from 100 to 88% and mobile phase B from 0 to 12% at a flow rate of 0.3 mL/min. The temperature of the HPLC column was set at 30°C. Under these chromatographic conditions, D and I were detected at 8.95 and 9.90 min, respectively. The mass spectrometer was operated in the positive-ion mode with the following spectrometric parameters: capillary voltage, 3.20 kV; sample cone voltage, 55 V; ion energy, 0.5 eV; amplifier voltage, 650 V; temperature of the desolvation, 400°C; and temperature of the source, 120°C. *Bottom*, *B*: quantification of D and I was achieved by a single ion record of D and I molecular ions, both at a mass/charge ratio of 526.25 (two isomeric molecules), which were produced from the LC/MS analysis. Peak areas of the single ion record obtained by D and I standards gives good linearity between 0.05 and 5 ng.