Urinary desmosine excretion in acute exacerbations of COPD: a preliminary report


*Laboratorio di Biochimica e Genetica della Clinica di Malattie dell’Apparato Respiratorio, †Dipartimento di Biochimica, Servizio Biometria ed Epidemiologia Clinica, IRCCS Policlinico San Matteo, Università degli Studi di Pavia, Departments of Medicine, Boston University School of Medicine and VA Boston Healthcare System, Boston MA, U.S.A.

Abstract Desmosine (DES) is an elastin-derived, cross-link amino acid, which is not metabolized; hence, its urinary levels reflect elastin breakdown. We hypothesized that elastin degradation should increase as a result of increased lung inflammation during an acute exacerbation of COPD and should decrease after recovery. To test this hypothesis we measured DES in three urine samples from nine COPD subjects during the first 5 days of an acute exacerbation and at 2 months after recovery. We also measured forced expiratory volume in 1 sec (FEV1) to monitor the effects of the exacerbation on ventilatory function. The mean (sd) FEV1 was 45 (15)% predicted during the exacerbation and 57·8 (16)% predicted 2 months later (P=0·0001). The mean (sd) DES excretion was 25·3 (9) μg g−1 creatinine at day 1, 23·5 (9) at day 3 and 24 (9) at day 5 of the exacerbation. The mean (sd) urinary DES excretion 60 days after discharge was 20·9 (7) μg g−1 creatinine (P=0·049) in comparison with the mean of the three acute-phase values. The size of the increase in desmosine excretion during exacerbation is small, 3·2 μg g−1 creatinine or 16% of the recovery desmosine value. We conclude that there is a small but statistically significant increase in lung elastin breakdown in the body during an acute exacerbation of COPD.

Keywords elastin; high performance capillary electrophoresis; micellar electrokinetic chromatography.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a destructive lung disorder characterized by chronic, partly reversible airflow limitation and the presence of chronic bronchitis or emphysema. Degradation of extracellular matrix components, mainly elastin, due to an imbalance between proteinases and their naturally occurring inhibitors is a key pathological feature of emphysema (1). Excess elastin degradation in the body results in excretion in the urine of the elastin-derived amino acids, desmosine (DES) (2). This cross-linked amino acid is unique to mature elastin and is not metabolized in the body. Urinary excretion of DES has been reported to be higher in patients with COPD, and in current smokers with normal lung function (3,4) than in never-smoking controls.

We recently applied a new separation technique, high-performance capillary electrophoresis (HPCE), in its micellar electrokinetic chromatographic (MEK C) modality, to the quantification of urinary DES in subjects with a variety of chronic destructive lung diseases, including subjects with COPD, disseminated bronchiectasis of unknown origin, pulmonary emphysema associated with inherited deficiency of α1-anti-trypsin (AAT) and cystic fibrosis (4).

In a preliminary study, we noted that COPD patients with exacerbations excreted more elastin cross-links than patients with stable COPD (4). We reasoned that elastin degradation increased due to increased inflammation in the lungs during an acute exacerbation of COPD, which resulted in increased urinary DES excretion. If this were so, urinary DES values should decrease after recovery from the exacerbation. To test this hypothesis we collected three urine samples from each of nine COPD subjects during the first 5 days of an acute exacerbation and we collected a fourth urine sample at 2 months after recovery. We also measured forced expiratory volume in 1 sec (FEV1) serially during the study to monitor the
effects of the exacerbation on ventilatory function. Our study shows a small, but statistically significant decrease in urinary DES excretion at 2 months, in comparison with values during the acute exacerbation. This is accompanied by a significant increase in FEV1 at 2 months compared to the acute exacerbation.

MATERIALS AND METHODS

Design of the study

This investigation was designed as a longitudinal study, which was approved by the hospital’s Ethical Committee. All patients gave their informed consent prior to entering the study.

Patients

We enrolled nine consecutive patients admitted to the Clinica di Malattie dell’Apparato Respiratorio, San Matteo Hospital, because of acute exacerbation of COPD. These subjects belong to a cohort of patients regularly seen in the outpatient clinic of the same department, in whom the diagnosis of COPD had been previously established according to ATS criteria (5). All patients were male former smokers.

Definition of exacerbation and selection of patients

Following the criteria of Anthonisen et al. (6), we diagnosed an acute exacerbation of COPD by the presence of two or three of the symptoms of increased dyspnoea, increased sputum volume and development of sputum purulence. The initial assessment of patients was performed in the emergency room (ER) of San Matteo Hospital. In our study we enrolled only patients admitted because of a moderately severe exacerbation, thus excluding patients with a mild exacerbation who were immediately discharged from the ER and treated at home. We also excluded those with a severe exacerbation, requiring mechanical or non-invasive ventilation (7). Patients who had had another episode of acute exacerbation within the previous three months or had already received oral corticosteroids at the time of admission were excluded from the study.

Patients’ assessment and treatment

At the time of hospitalization, the patients were submitted to routine assessment, including blood gas analysis, chest X-ray (to exclude pneumonia) and sputum culture. The patients received aminophylline every 12 h, methylprednisolone 30 mg every 24 h i.v., nebulizations of oxitropium bromide and beclomethasone every 8 h, low flow oxygen supplementation via nasal cannula and an 8-day course of oral amoxycillin/clavulanic acid 1 g every 12 h (in one case the antibiotic therapy was changed to i.v. cefotaxime 1 g every 12 h after 48 h because of isolation from sputum of K. pneumoniae resistant to amoxycillin/clavulanic acid). All associated conditions were treated appropriately. Pulmonary functions tests were performed as soon as the general and respiratory condition of the patients allowed, but in all cases within the first 4 days of the hospital admission. Table 1 reports the clinical characteristics of the patients enrolled in the study.

Outcome of the exacerbation and follow-up

The patients’ discharge was decided on the basis of remission of symptoms and signs used to define the episode of exacerbation. Still-needed treatments were switched from the intravenous to the oral route; oral methylprednisolone was tapered and definitively withdrawn within 3 weeks after discharge. All patients were telephoned every 2 weeks to inquire about their condition. A follow-up examination was performed 60 days after discharge. Stability of their respiratory state was checked by a brief questionnaire and pulmonary function tests were performed.

Timing of urine collection

Three urine samples were collected during the hospitalization: one within the first 24 h (T1), the others on the third (T3) and fifth (T5) days. At the 2-month follow-up visit, patients delivered a urine sample collected during the 12 h prior to the appointment (T60).

Table 1. Characteristics of the patients with acute exacerbation of COPD*

<table>
<thead>
<tr>
<th>No</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Smoking history (pack-years)</th>
<th>FEV1 (%) pred.</th>
<th>FVC (%) pred</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Male</td>
<td>69 (5)</td>
<td>164 (7)</td>
<td>71 (5)</td>
<td>35 (7)</td>
<td>45 (5)</td>
<td>59 (2)</td>
</tr>
</tbody>
</table>

*Data are expressed as mean (SD). Six subjects had three of the defining symptoms of an exacerbation (6); three subjects had two of the three symptoms.

†Post-bronchodilator data obtained within the fourth day of hospitalization.
Biochemical evaluation

Chemicals

Sodium phosphate (used as a background electrolyte) was purchased from Bio-Rad (Richmond, CA, U.S.A). Doubly distilled water was obtained from a Millipore (Bedford, MA, U.S.A.) Milli-Q purification system. All other chemicals were of analytical grade and were used without further purification.

Urine collection and treatment

Urine samples were collected into sterile plastic bottles, starting in the morning, until a volume of 100 ml was obtained, which was stored at −20 °C until processed. Each sample was concentrated under reduced pressure using a heated rotary evaporator (50 °C). A sample of urine (0-8 ml) was then ultracentrifuged at room temperature with microconcentrators Microcon 30 (Millipore) and transferred to hydrolysis pyrex tubes, evaporated to dryness and hydrolysed in vacuo with HCl 6N at 106 °C for 24 h. Hydrolysed samples were lyophilized and the residue dissolved in deionised water (repeated twice in order to remove HCl totally). Finally, samples were taken up with 0-4 ml of distilled water and submitted to HPCE. The content of DES was expressed as mg g⁻¹ creatinine.

Capillary electrophoretic instrumentation and running conditions

All runs were performed using a Bio-Rad Biofocus 3000 system equipped with a high-speed UV-Vis scanning detector. Fused silica capillaries of 57 cm (50 cm effective length from inlet to detector) × 50 μm ID were obtained from Beckman (Palo Alto, CA, U.S.A.). The procedure followed was essentially as described previously (8), with the modification subsequently adopted (4).

Statistical analysis

Data are presented as mean and standard deviation (SD). Normality of data distribution was assessed by the Shapiro–Wilk test. Repeated measure MANOVA was used to test for statistically significant changes over time for DES and t-test for paired data of FEV₁. A P-value of less than 0.05 was considered to be statistically significant. All tests were two-sided. Analyses were performed by the software STATA (StatCorp, 1999; Stata Statistical Software release 6-0 College Station, TX, U.S.A.).

RESULTS

Urinary excretion of elastin cross-links changed little during the first 5 days of hospital admission: mean (sd) DES excretion was 25.3 (9) μg g⁻¹ creatinine at T₄, 23.5 (9) at T₃ and 24 (90) at T₅. All comparisons were non-significant.

Pulmonary function tests, performed on the fourth day of the admission, revealed moderate airflow obstruction. Mean (sd) FEV₁ was 45 ± 15% predicted and the mean (sd) forced vital capacity (FVC) was 59 (12)% predicted.

All subjects enrolled in this study recovered from their acute exacerbation of COPD. Symptoms subsided promptly, and patients were discharged after a mean (sd) hospital stay of 7 (2) days. After discharge, patients were telephoned every 2 weeks, to gain information about their condition and adherence to treatment. In particular, oral corticosteroids were withdrawn from all subjects within 3 weeks after discharge.

At the follow-up appointment, 60 days after discharge, all subjects were in stable clinical condition and none had experienced a further episode of acute exacerbation, as determined by the questionnaire. Pulmonary function tests showed a mean (sd) FEV₁ of 57-8 (16)% predicted and a mean (sd) FVC of 73 (13)% predicted. The difference in FEV₁ values between exacerbation and follow-up was highly significant (P=0.00001).

The mean (sd) urinary excretion of DES, as determined at T₆₀, was 20-9 (7) μg g⁻¹ creatinine. The difference between T₆₀ and the mean of the three values obtained during the exacerbation was statistically significant (P=0.049). Figure 1 shows a plot of urinary excretion of DES and of FEV₁ during the study period.

DISCUSSION

Emphysema is one of the critical elements for defining COPD. Although the precise details of the events in the alveolar walls are poorly understood, there are three main hypotheses of its pathogenesis. These are more

![Graph showing time course of desmosine (----) and FEV₁ (--). Points represent mean values (sd). The shaded area represents the mean ± 1 · 96 sd of urinary DES in 12 healthy never-smokers (4). Note that mean values of DES in this study are above the range of normal at all time points. Values on y-axis are μg g⁻¹ for DES and % predicted for FEV₁.](image-url)
overlapping than exclusive and include the elastase—anti-
 elastase imbalance hypothesis (I), the oxidant—anti-oxi-
dant imbalance hypothesis (9) and the infection hypoth-
osis (10). This study does not specify between these three
 hypotheses. It suggests that excess elastin degradation
 occurs in an acute exacerbation of COPD because of in-
 creased inflammation in the lungs. Elastin degradation
 could be due to elastase—anti-elastase imbalance, to ox-
dant—anti-oxidant imbalance in the lungs or to infection;
 we do not distinguish among inflammation due to
 viruses, bacteria or chemical irritation, as from urban
 air pollution.

 Previous studies have shown that urinary DES is ele-
vated in subjects with COPD in comparison to both
 never-smokers and smokers without airflow obstruction
 (3). Note in Fig. I that mean values of DES in this study are
 above the range of normal at all time points. We hoped in
 the current study to determine whether elastin degra-
dation was increased during an acute exacerbation
 of COPD as compared with the stable state. Accordingly,
 we measured cross-link excretion during the acute phase
 of an acute exacerbation (days 1—5). Since baseline ur-
 inary DES values were not available on our subjects, we
 assumed that urinary DES/IDES excretion during the
 chronic phase (day 60) would be a reasonable reflection
 of the pre-exacerbation, stable state. We also measured
 the FEV1 during the acute and late phases of the study.
 We reasoned that the FEV1 would be decreased during
 the acute phase of the study as a result of increased in-
 flammation in the lungs and should be restored during
 the chronic phase as the inflammation accompanying
 the exacerbation subsided. Two recent reports support
 the behaviour of the FEV1 in this condition. Hill et al. (II)
 compared II patients with homozygous AAT deficiency
 and II control patients with COPD and normal AAT lev-
 els during an exacerbation. All patients received 14 days
 of antibiotic therapy; none received oral corticosteroids.
 The concentrations of sputum myeloperoxidase activity
 (which served as an assessment of neutrophil influx), spu-
tum elastase activity, IL-8 and LTB4 concentrations all fell
 significantly by the third day of the exacerbation and had
 reached a nadir by day 14. At presentation, sputum elas-
tase activity, IL-8 and LTB4 concentrations were lower in
 the control COPD patients than in the AAT-deficient
 COPD patients; the time course of improvement was si-
milar in the two groups. In the AAT-deficient group,
 mean (se) peak expiratory flow rate was 236.4 (41.4)
 l min1 at presentation and rose by 11.9 per cent of base-
 line (P < 0.005) by day 14. Peak flow rate results were si-
milar in the usual COPD group.

 Bhowmik et al. (12) studied ventilatory function, spu-
tum cells and cytokines in 57 patients with moderate to
 severe COPD during the stable state and during an ex-
 acerbation of their disease. Median values of IL-6, IL-8,
total cell count, total neutrophils, total eosinophils and
total lymphocytes were higher during exacerbation than
 in the stable state, but the only significant difference
 (P < 0.05) was for IL-6. The mean (sd) FEV1 was 1.07
 (0.26) l during the stable state and fell to 0.99 (0.31) l
 (P > 0.05) at the onset of the exacerbation. The mean
 peak expiratory flow rate data, 239 (78) l min1 and 225
 (73) l min1, respectively, were statistically significantly
 different (P < 0.05).

 By 60 days after discharge from hospital, all of our pa-
tients showed sustained clinical and functional recovery
 from the acute exacerbation, as witnessed by the ab-
sence of symptoms and marked improvement in FEVi.
 Thus, we have shown in this study that there was a small,
 but statistically significant decrease in urinary DES va-
 lues between the acute and chronic phases of the study
 and these changes were accompanied by an increase in
 ventilatory function. Hill et al. (II) showed stability of in-
fammatory parameters by day 14. Indeed in their data,
 values of these parameters were higher at day 28 than
 at day 14.

 It is widely accepted that recurrent exacerbations ac-
celerate airway and parenchymal damage in COPD (13),
 we noted only a modest increase in urinary DES excre-
tion between the acute and chronic phases —3.2 μg g1
 creatinine or 16% of the chronic phase value. However,
 over a period of years, even slight increases in elastin de-
gradation during an exacerbation may cause a sustained
 injury. It remains to be determined how long the increase
 in urinary DES excretion lasts after an exacerbation and
 whether there is indeed a complete recovery to baseline
 excretion rate.

 Previous papers have failed to demonstrate a correla-
tion between urinary DES levels and FEVi measured at a
 single time point in a group of patients (4,14). These ob-
 servations are not surprising since ventilatory function
 at any one point in time is determined by the amount of
 elastin degradation that has occurred in the past over an
 extended period of time—a period of years. On the
 other hand, it makes perfectly good sense that over a
 period of 2 months after an acute exacerbation of
 COPD, changes in lung inflammation, urinary cross-link
 excretion and ventilatory function should mirror one an-
 other.

 In mice, after an acute elastolytic injury, more than
 half of the desmosine-containing peptides removed from
 the lungs are sequestered by the kidney and then slowly
 released into the urine over a period of several days (15).
 The role of the kidney in the kinetics of elastolysis in
 stable or acute conditions in humans is undetermined.
 However, we speculate that in our study, any sequestra-
tion of DES in the kidney should have been over by 60
days after the acute exacerbation.

 Because of the great expense of evaluating new drugs
 for COPD using rate of change of FEV1 as the outcome
 variable (16), there is much interest in the possible use of
 measurement of urinary elastin cross-links as a surrogate
 marker of elastin degradation in the lungs for drug
development in COPD (4, 17, 18). A recent report failed to demonstrate that short-term AAT replacement therapy in AAT-deficient patients was able to reduce elastin breakdown (18) despite baseline elevation of urinary DES levels in these patients. However, the failure of an effect could have been due to a low dose of augmentation therapy with AAT or elastolysis could have been due to macrophage-derived metalloelastase, which is not inactivated by AAT.

The changes in urinary DES between the acute and chronic phases of the study were small and the variability of the DES values is large (coefficient of variation for the six T1, T3, T5 and T60 groups was about 35%). Hence, the data reported here must be considered preliminary and larger studies should be done. Nevertheless, our results raise the question of whether anti-elastase or anti-oxidants might be administered to protect the lungs during an acute exacerbation. While studies of elastase, oxidants or inflammatory mediators in sputum or bronchoalveolar lavage might be used to determine the efficacities of such drugs, urinary DES measurements are a more definitive measure of lung destruction and should be used as well.

Acknowledgements

Supported by the Italian Ministry of Health, CF projects, law 548/93, with a grant given to IRCCS Policlinico San Matteo, Pavia, Italy.

REFERENCES