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Exhaled nitric oxide, but not serum nitrite and nitrate, is a marker of interstitial lung disease in systemic sclerosis

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ABSTRACT

Nitric oxide metabolites (NOx) in serum, and alveolar concentration of NO (CA_{NO}), are markers of inflammation and alveolitis, respectively, in systemic sclerosis (SSc). We prospectively evaluated the usefulness of both NOx and CA_{NO} to assess lung involvement and skin fibrosis in SSc. Serum NOx, and CA_{NO} measured by two different methods, namely the two-compartment (2CM) and the "trumpet" models (TM), were concomitantly assessed in 65 patients with SSc and 17 healthy controls. Whilst serum NOx remained comparable between groups, CA_{NO} were significantly higher in SSc patients ($n = 65$, 6.7 ppb; 4.8–9.7 and 5.9 ppb; 3.9–8.9) as compared with controls ($n = 17$, 3.0 ppb; 2.0–3.8 and 1.8 ppb; 1.1–2.9, $p < 0.001$, $p < 0.001$) using the 2CM and the TM, respectively). CA_{NO} from SSc patients with interstitial lung disease (ILD) ($n = 26$, 8.6 ppb; 6.5–10.9 and 8.5 ppb; 5.9–10.7) or pulmonary arterial hypertension ($n = 12$, 7.3 ppb; 6.5–10.4 and 6.9 ppb; 5.4–9.9) were significantly higher as compared with patients without ILD ($n = 27$, 4.9 ppb; 3.8–6.5 and 4.7 ppb; 2.8–5.7; $p < 0.001$ and $p < 0.001$) using the 2CM and the TM, respectively). CA_{NO} assessed either by the 2CM or the trumpet model were directly related to the extent of ILD and inversely related to DLCO. There was no correlation between NOx and ILD, or DLCO. Neither CA_{NO} nor NOx was correlated with skin fibrosis and no relationship was found between CA_{NO} and NOx.

Alveolar concentration of NO, but not serum NOx, closely correlates with the extent of ILD in patients with systemic sclerosis. Neither parameter, however, is related to skin fibrosis.

Introduction

Systemic sclerosis (SSc) is a connective tissue disease characterized by vascular endothelial damage and fibrosis. Endothelial damage is the primary event that eventually leads to major vascular disorders in SSc, such as Raynaud phenomenon and pulmonary arterial hypertension. Excessive fibrosis, that takes place in many organs, including the lung, leads to interstitial lung disease (ILD) in SSc, which considerably worsens the prognosis of the disease [1]. Interstitial lung disease eventually causes restrictive lung function and reduces gas lung transfer [2]. The loss of lung volume is associated with increased morbidity and respiratory failure is now the main cause of mortality in patients with SSc [3]. ILD, associated or not with connective tissue disease, could be the result of an ongoing lung inflammation, as suggested by histological findings from lung biopsies of patients with SSc [2,4] showing the presence of inflammatory cells associated with diffuse vascular damage and excessive production of extra cellular matrix by myofibroblasts [5]. Due to its invasive nature, broncho-alveolar lavage (BAL) cannot be performed on a regular basis during follow-up of patients with scleroderma lung disease. Furthermore, the usefulness of data derived from BAL fluid analysis in the management of scleroderma lung disease is still a matter of debates [6–8]. Consequently, it is not easy to assess ongoing inflammation in the alveoli, neither it is possible to evaluate whether ILD is presently active in SSc patients. Lung HRCT can detect ILD in various stages of the disease, before the occurrence of respiratory failure, but structural abnormalities, including ground glass opacity and/or reticular opacity seen on HRCT, vary little with treatments and loosely reflect alveolar inflammation that usually precedes impaired pulmonary lung diffusing capacity. There is, therefore, a need for more dynamic and accurate inflammation markers capable of detecting the presence, and possibly the deterioration of ILD in SSc.

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Nitric oxide (NO) is an ubiquitous cell mediator that not only plays an important role both as a physiological modulator of pulmonary vascular tone, and a pathological pro-inflammatory mediator implicated in many lung disorders [9]. Thus, NO is a dual-purpose marker that can reflect either the extent of endothelial damage or the presence of lung inflammation preceding the occurrence of fibrosis in SSc. There are different ways to measure the amounts of NO produced by the organism. Serum total level of nitrite and nitrate (NOx) reflects indirectly NO output from food intake, bacterial conversion of nitrate, and the amount of NO produced in physiological condition in addition to the amount of NO produced by the inducible NO synthase (iNOS). In SSc, variations of NOx serum levels have been reported to be associated with clinical subsets of disease [10]. The interpretation and the clinical values of serum levels of NO metabolites, i.e. nitrite and nitrate (NOx), in patients with SSc are still debated [10–12]. Previous reports showed that the serum levels of NOx were either increased [10,13] or decreased [14] in SSc patients as compared to healthy controls. It has also been found that NO output measured in the mixed exhaled air was increased in SSc patients with ILD [15–17]. Using the two-compartment model to partition the source of exhaled NO into airway conduct flux ($J'aw_{NO}$) and alveolar concentration of NO (CA_{NO}) [18], we [19] and others [20] have shown that the increase of CA_{NO} was directly related to the severity of lung disease in SSc patients.

Taking into account the trumpet shape of the airway cross-sectional area and the axial diffusion of NO, Condorelli et al. have recently proposed another technique using constant flow exhalations to characterize proximal and peripheral NO exchange in healthy subjects [21]. It is likely that this new and so-called "trumpet" model provide more accurate assessment of bronchial and alveolar NO output than the earlier, i.e. two-compartment, model in healthy subjects. However, comparisons between these two models have not yet been made in diseased patients, in particular patients with SSc-related ILD. Neither it is known whether NOx or CA_{NO} estimated by the two models can better reflect pulmonary vascular disease and lung fibrosis, or skin involvement, in SSc. Therefore, the aim of this study was to compare the accuracy of NOx and that of CA_{NO} to assess pulmonary artery hypertension (PAH), ILD and skin fibrosis in patients with SSc.

Methods

Subjects

Between November 2005 and April 2007, 65 patients (58 women, 7 men, median; first and third quartiles 58.5 years; 50.9–63.9), fulfilling the American College of Rheumatology criteria of SSc [22] and 17 healthy controls (12 women, 5 men, 50.0 years; 33.3–60.0) were included successively in this prospective study at Saint-Antoine Hospital, academic referral centre of SSc in France. Median duration of the disease (defined as the number of years after the first symptom attributable to SSc) was 10.2 years; 4.1–19.3. Twenty-six patients had diffuse (dSSc) and 39 had limited form of SSc (lSSc) according to LeRoy classification subset [23]. Fourteen patients were current smokers, however, they refrained from smoking 72 h before exhaled NO measurement. Fifteen patients were treated with low doses (less than 10 mg/day) of corticosteroids, 8 (out of 15) also took immunosuppressive therapy, but none took NO donors. After giving their informed consent, all patients underwent pulmonary function tests (PFTs), and echocardiogram, pulmonary HRCT. Exhaled NO was measured by the two models and sera were collected from all patients and healthy controls on the same day, and sera were stored at −80 °C until assay for NOx measurement.

Pulmonary high resolution computed tomography

Interstitial lung disease was deemed present if pulmonary HRCT demonstrated compatible changes in reticular or air space opacities. These changes included ground glass attenuation, defined as a hazy increase of lung parenchyma attenuation, and reticular fibrosis, defined as lobular septal thickening and subpleural honeycomb change. To assess the extent of ILD in individual cases, ground glass and fibrosis scores were estimated in each lobe. Five different grades were given according to the importance and the extent of lung opacities [24]. Grade 0: meant no abnormality, grade 1: less than 5% of the lobe, grade 2: 6–25% of the lobe, grade 3: 26–50% of the lobe, grade 4: 51–75% of the lobe, and grade 5: 76–100% of the lobe.

Echocardiogram

All patients underwent echocardiogram (Vivid® 7 G.E medical systems, Norway). To estimate systolic pulmonary artery pressure (sPAP), the maximal transtricuspid pressure gradient was calculated using simplified Bernouilli equation [25]. To calculate right ventricular systolic pressure which was equal to sPAP, 10 mmHg, as an estimate of right atrial pressure, was added to the pressure gradient. Patients with a right ventricular systolic pressure of $\geqslant$40 mmHg on echocardiogram underwent right heart catheterization. Pulmonary artery hypertension was defined by mean pulmonary pressure higher than 25 mmHg at rest or higher at 30 mmHg in exercise [26].

Lung function measurement

Pulmonary function tests (PFT), assessing forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), lung diffusion for carbon monoxide (DLCO), alveolar volume (VA), and blood gas measurements were performed (MasterScreen® Body, VIASYS Healthcare GmbH, Hoechberg, Germany) according to the American Thoracic Society/European Respiratory Society recommendations on clinical pulmonary function testing [27]. All parameters were expressed as percentage of predicted values.

Partitioned exhaled NO measurement

NO was measured using a chemiluminescent analyzer (EndoNO 8000®, SERES, Aix-en-Provence, France), according to validated method for the online measurement of the exhaled NO concentration (F_{ENO}) in adults [28]. After full inspiration from room air with ambient NO levels less than 20 part per billion (ppb), the subject exhaled against a positive pressure that was constantly kept between 5 cmH_2O (lower limit) and 20 cmH_2O (upper limit) to generate expiratory flow rates (V'_E) of 100, 150 and 200 ml/s. For each V'_E, the elimination rate of NO (V'_{NO}) was calculated ($V'_{NO} = V'_E \cdot F_{ENO}$). F_{ENO} is inversely related to V'_E, whereas V'_{NO} varies directly as a function of V'_E. At the flow rate >50 ml/s, the latter relationship is linear and can be expressed as $V'_{NO} = V'_E \cdot F_{ENO} = CA_{NO} \cdot V'_E + J'aw_{NO}$ [18,29]. For each patient, the R^2 values of the relationship between F_{ENO} and V'_E were calculated.

Trumpet model with axial diffusion

For all patients and healthy controls, we estimated the $J'aw_{NO}$ and the CA_{NO} according to the model described by Condorelli et al. [21], taking into account the trumpet shape of airway tree and the axial diffusion of NO and simplifying the mathematical equations of the dynamic exchange of NO in the respiratory system. We used the linear relationship to express the variation of V'_{NO}, (pl/s) as a function of V'_E (ranging from 100 ml/s to 250 ml/s):