

Mechanisms of increased epithelial lung clearance

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Mechanisms of increased epithelial lung clearance of DTPA in diffuse fibrosing alveolitis. S. Labrune, Th. Chinet, M.A. Collignon, L. Barritault, G.J. Huchon. ©ERS Journals Ltd 1994.

ABSTRACT: The aim of this study was to elucidate possible mechanisms of increased epithelial lung clearance in diffuse fibrosing alveolitis (DFA).

We investigated the relationships between epithelial lung clearance as assessed by the clearance of aerosolized ^{99m}Tc -diethylene-triamine-penta-acetic acid (RC-DTPA), luminal alveolitis as assessed by bronchoalveolar lavage, and pulmonary function, in 30 nonsmokers with DFA. In 14 of these patients, RC-DTPA and lung function were determined before and during therapy with prednisolone (0.5 mg·kg⁻¹ daily).

RC-DTPA was higher in patients with DFA ($4.45 \pm 2.50\% \cdot \text{min}^{-1}$) than in normal subjects ($1.18 \pm 0.31\% \cdot \text{min}^{-1}$). RC-DTPA did not correlate with the number of alveolar neutrophils, but correlated positively with the number of alveolar lymphocytes, and negatively with vital capacity (VC). RC-DTPA decreased from 6.1 ± 2.8 to $3.8 \pm 1.9\% \cdot \text{min}^{-1}$ with prednisolone. RC-DTPA before prednisolone correlated positively with the prednisolone-associated improvement in VC.

We conclude that in patient with DFA, RC-DTPA is increased, and decreases but does not return to normal with corticosteroid therapy. Our data suggest that in DFA the increase in RC-DTPA could be related to the recoil-induced stretch of the respiratory epithelium and to alveolar lymphocytic inflammation.

Eur Respir J., 1994, 7, 651–656.

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Keywords: Alveolar lymphocytosis
inflammation
lung recoil
pulmonary function

Received: April 20 1993
Accepted after revision November 29 1993

This work was supported by a grant from Association pour l'Étude de la Respiration et de l'Environnement (AERE).

Diffuse fibrosing alveolitis (DFA) is characterized by the infiltration of alveolar structures by inflammatory cells, that leads to progressive fibrosis [1–5]. The extent of inflammation, (which is potentially reversible) and fibrosis (which is not) may be assessed either directly by open lung biopsy [6–10], or indirectly by bronchoalveolar lavage (BAL), which provides information on luminal alveolitis [4, 11–16]. Pulmonary function tests (PFT) do not differentiate between the relative contributions of cellular infiltration and fibrosis to functional impairment [5]. The epithelial lung clearance of aerosolized ^{99m}Tc -diethylenetriamine penta-acetate (RC-DTPA), which is mainly an assessment of the permeability of the epithelium of the terminal respiratory units to solutes [17], has been found to be increased in various conditions characterized by the presence of inflammation and/or changes in lung volumes, including mediator release [18, 19], increase in lung volumes [20, 21], and DFA [20, 22, 23]. However, causes of the increase in RC-DTPA in DFA are unknown. Therefore, we investigated possible mechanisms of increased RC-DTPA in patients with DFA by studying: 1) the relationships between RC-DTPA and pulmonary function and luminal alveolitis as assessed by BAL; and 2) changes in RC-DTPA and pulmonary function with corticosteroid therapy.

Materials and methods

Patients

At the time of inclusion in the study, the diagnosis of DFA was made in 30 untreated nonsmokers on either open lung biopsy findings (n=10), and/or on evidence of progressive dyspnoea, bilateral crepitations over the lungs, a pattern suggesting interstitial pneumonitis on chest radiogram, a decrease in all lung volumes, and no evidence of environmental or occupational exposure, hypersensitivity pneumonitis or left ventricular failure (n=20) [2, 3, 5]. DFA was associated with a connective tissue disease in eight patients (three rheumatoid arthritis, four dermatopolymyositis and one mixed connective tissue disease), and was considered to be idiopathic in 22 patients. PFT, RC-DTPA and BAL measurements were performed in that order, within a week, in all patients at the time of inclusion in the study. All patients subsequently received prednisolone (0.5 mg·kg⁻¹ daily). In 14 of the 30 patients, RC-DTPA and lung function measurements were repeated 2–6 months after initiating the corticosteroid therapy. Thirty eight healthy nonsmokers had RC-DTPA measurements, as a control group.

Pulmonary function tests (PFT)

Forced expiratory volume in one second (FEV_1) and vital capacity (VC) were measured with a spirometer whilst the patient was in the seated position, and the FEV_1 /forced vital capacity (FVC) ratio was calculated. The total lung capacity (TLC) was derived from the measurement of the functional residual capacity using the helium dilution method. Results were expressed as percentage of the predicted values from the European Coal and Steel Community Survey [24]. Diffusing capacity for carbon monoxide (DLCO) was measured by the breathholding technique, and was expressed as a percentage of predicted values based on age and alveolar volume. Arterial oxygen tension (PaO_2) and arterial carbon dioxide tension ($PaCO_2$) were measured, and the alveolar-arterial oxygen tension difference ($(A-a)DO_2$), was calculated using the alveolar equation during steady-state conditions, on the assumption of a respiratory gas exchange ratio of 0.8.

RC-DTPA

RC-DTPA was measured as described previously [23]. ^{99m}Tc was eluted from a ^{99}Mo generator (CEA, Oris-Cis generator, CEA, Saclay, France), and diluted in saline; 50 mCi of sodium pertechnetate was introduced into a vial containing 3.3 mg of DTPA to produce ^{99m}Tc -DTPA. The radionuclide preparation obtained more than 95% of bound ^{99m}Tc -DTPA as determined by paper chromatography. The aerosol (0.8 μm mass median aerodynamic diameter (MMAD), 2.4 GSD) of ^{99m}Tc -DTPA was generated by a jet nebulizer (Venticis, Oris, CEA, France) and inhaled by subjects whilst supine. The nebulizer was connected to a separator (Venticis, Oris, France) that removed the largest particles ($>2 \mu m$). The subjects inhaled the aerosol for 3 min at their normal tidal volume, through a nonbreathing T-piece system, until about 2.5 mCi were retained in the lungs. Counts of radioactivity were recorded in 30 s frames from the entire thorax in the posterior projection, using a gamma-scintillation camera (PHO/Gamma HP3, Nuclear Chicago, Chicago, IL, USA). At the end of the experiment, the peak counts at the outset were displayed on a screen and peripheral portions of the lungs were selected for analysis. Counts were corrected for radionuclide decay and plotted on a semi-logarithmic scale against time. Regression line of the counts from both lungs *versus* time was calculated by the least squares method during the 10 min period after the activity peak. RC-DTPA was defined as the negative slope of this line expressed as the percentage decline in counts per minute.

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) was performed as described previously [25]. The fibroscope was introduced by the nasal or oral route, in patients sitting upright, who had received premedication of 1 mg of atropine sulphate and who had been given locoregional anaesthesia with 2% xylocaine. After examination of the bronchial tree, BAL was performed in a subsegmental bronchus of the middle lobe. The lavage was carried out by instillation

of 300 ml sterile saline solution (0.15 M NaCl) at 37°C. The liquid was introduced through the lateral canal of the fibroscope in 100 ml fractions. Each fraction was recovered after instillation by simple siphoning, initiated by the application of a negative pressure equal to 5–10 cmH_2O .

The liquid was recovered in a sterile glass flask, siliconized to reduce the adherence of cells to the walls of the flask. The volume of fluid recovered was measured, and the liquid was filtered over a sterile gauze to eliminate the mucus. After homogenization, a small volume was removed for total and differential cell counts. After agitation of the fluid, the counts were made on a haemocytometer within 30 min of the lavage. For morphological examination, slide preparations were made in a Shandon cytocentrifuge using a volume of fluid containing approximately 30,000 cells and spinning at 300 $\times g$ for 10 min. After fixation in methanol, the preparations were stained with May-Grünwald stain. Differential counts were made from counts of 1,000 cells. The upper limits of control values were: total cell count 1.2×10^6 cells·ml⁻¹ of fluid recovered; macrophages 0.98×10^6 cells·ml⁻¹; neutrophils 4%; eosinophils 1%; lymphocytes 12%; mast cells 0.5% (excluding epithelial cells from the calculation).

Statistical analysis

Results are reported as mean \pm SD. Comparisons between means were made using paired and non-paired t-tests as appropriate. Relationships between RC-DTPA, PFT, and bronchoalveolar cell counts were analysed by Spearman's rank correlation coefficient. A $p < 0.05$ was considered significant [26].

Results

PFT, RC-DTPA and BAL

In the group of patients with DFA, RC-DTPA was higher ($4.45 \pm 2.5\% \cdot \text{min}^{-1}$, range 0.5–12%·min⁻¹) than in

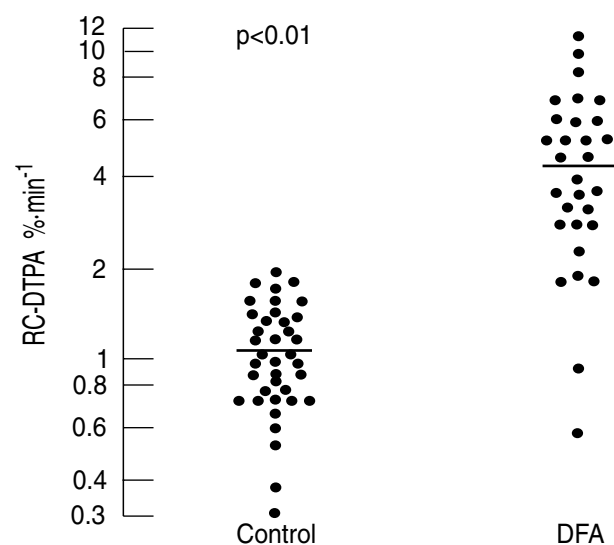


Fig. 1. — Individual values of the respiratory clearance of ^{99m}Tc -DTPA (RC-DTPA) in patients with diffuse fibrosing alveolitis (DFA) and in normal control subjects. The bars represent the mean values.

Table 1. – Pulmonary function tests, arterial blood gases and bronchoalveolar cell counts in 30 untreated patients with idiopathic pulmonary fibrosis

	Mean±SD	Range
Pao ₂ mmHg	72±11	45–90
kPa	9.6±1.5	6–12
Paco ₂ mmHg	40±4	31–54
kPa	5.4±0.5	4–7
TLC % pred	60±12	37–85
VC %pred	56±18	25–86
FEV ₁ % pred	59±20	31–95
FEV ₁ /FVC % pred	110±20	70–140
DLCO % pred	59±19	28–108
(A-a)Do ₂ mmHg	27±11	1.5–46
Lymphocyte count 10 ³ .ml ⁻¹	88±109	3.5–447
Neutrophil count 10 ³ .ml ⁻¹	30±41	1.8–192

Pao₂: arterial oxygen tension; Paco₂: arterial carbon dioxide tension; TLC: total lung capacity; VC: vital capacity; FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; DLCO: diffusing capacity of the lung for carbon monoxide; (A-a)Do₂: alveolar-arterial oxygen tension difference.

Table 2. – Spearman's rank coefficient (r_s) and significance level (p) for the correlation of RC-DTPA with pulmonary function tests and bronchoalveolar lavage variables

	r _s	p
Pao ₂	-0.036	NS
Paco ₂	-0.137	NS
(A-a)Do ₂	-0.203	NS
TLC	-0.190	NS
VC	-0.449	<0.02
FEV ₁	-0.417	<0.03
DLCO	-0.422	<0.03
Lymphocyte count	0.371	<0.05
Neutrophil count	-0.088	NS

RC-DTPA: respiratory clearance of aerosolized ^{99m}Tc-diethylene-triamine-penta-acetic acid. NS: nonsignificant. For further abbreviations see legend to table 1.

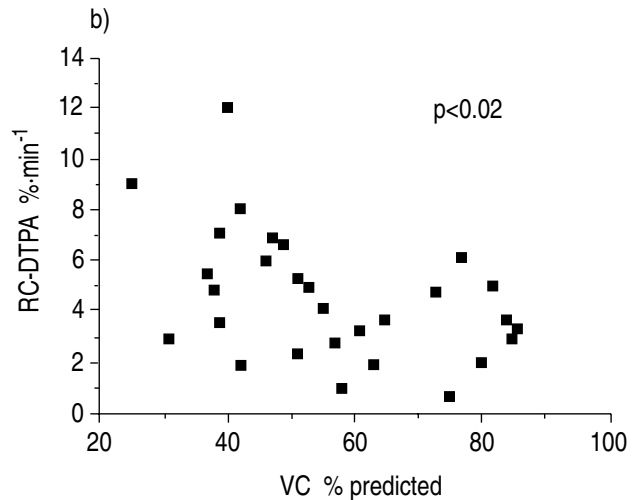
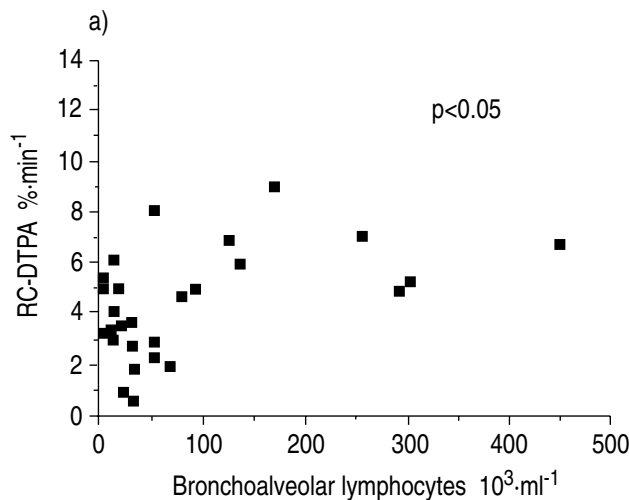


Fig. 2. – Correlation between respiratory clearance of ^{99m}Tc-DTPA (RC-DTPA) and: a) bronchoalveolar lymphocyte count; and; b) vital capacity (VC).

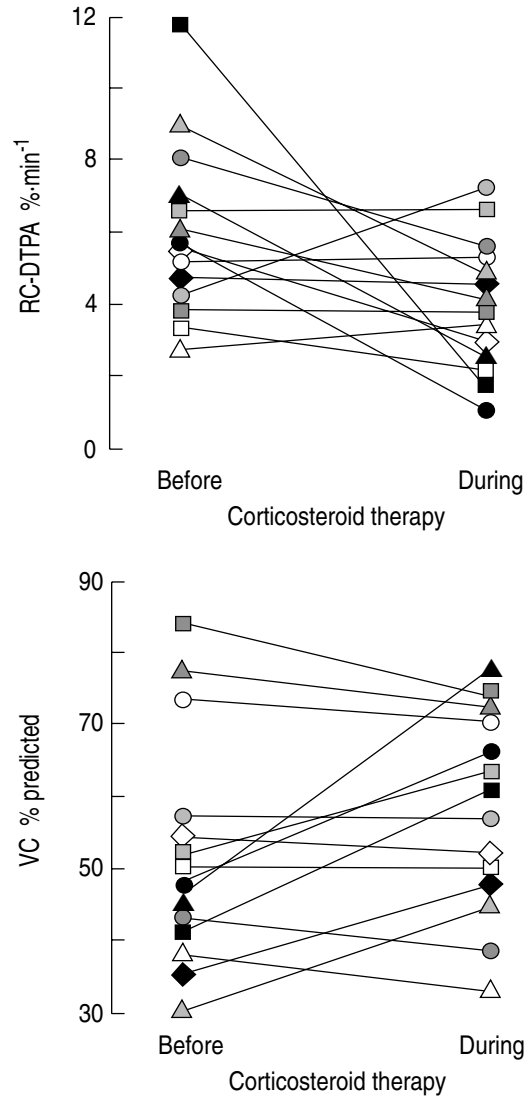


Fig. 3. – Individual values from 14 patients, shown by different symbols, of the respiratory clearance of ^{99m}Tc-DTPA (RC-DTPA) and of vital capacity (VC) before and during corticosteroid therapy.

the control group ($1.18 \pm 0.31\% \cdot \text{min}^{-1}$; $p < 0.01$) (fig. 1). Means, standard deviations and ranges of pulmonary function variables, arterial blood gases and bronchoalveolar cell counts are shown in table 1. Simple correlation coefficients of RC-DTPA with PFT and BAL data are shown in table 2. RC-DTPA ($\% \cdot \text{min}^{-1}$) correlated with both the alveolar lymphocyte count ($p < 0.05$) and the decrease in VC ($p < 0.02$). Figure 2a is the plot of RC-DTPA against number of alveolar lymphocytes. Figure 2b is the plot of RC-DTPA against VC. There was no correlation between PFT and BAL findings.

Effects of corticosteroid therapy

RC-DTPA decreased from 6.1 ± 2.4 to $4.0 \pm 1.7\% \cdot \text{min}^{-1}$ ($p < 0.03$), and VC increased from 50.4 ± 16.8 to $56.6 \pm 14.2\%$ predicted (NS). Individual values of RC-DTPA and VC before and during prednisolone therapy are shown in figure 3. The maximal change during corticosteroid therapy correlated with the value of RC-DTPA before treatment ($r = 0.60$; $df = 12$; $p < 0.02$) (fig. 4).

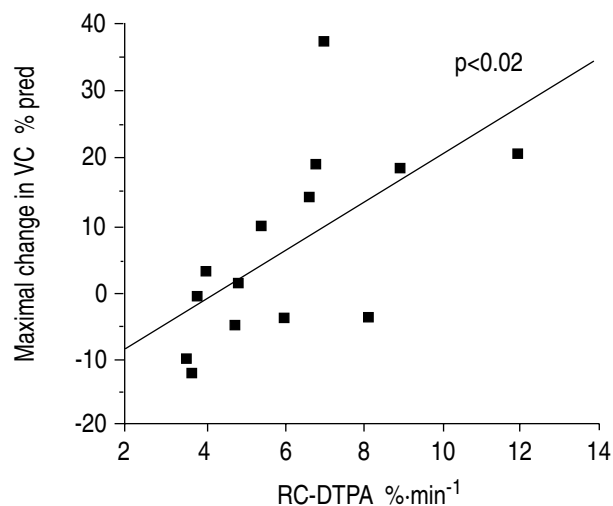


Fig. 4. — Individual values from 14 patients, and regression line of the maximal change in vital capacity (VC) between successive measurements during corticosteroid therapy and the respiratory clearance of ^{99m}Tc -DTPA (RC-DTPA) before treatment.

Discussion

This study confirms that RC-DTPA is increased in patients with DFA, and demonstrates that RC-DTPA decreases but does not return to normal during corticosteroid therapy, and is positively correlated with alveolar lymphocytosis and negatively correlated with VC.

Before discussing the significance of such results, a few methodological items need to be discussed, *i.e.* the RC-DTPA measurements and the assessment of pulmonary inflammation by BAL. Technical problems in the measurement of RC-DTPA have been extensively discussed elsewhere [17–19, 27, 28]; of particular importance are the effects of smoking, increase in lung volume, and correction for recirculation of the radiolabelled material.

None of the subjects included in this study smoked, and lung volumes were generally decreased in our patients. We did not correct for recirculation and we do not believe that correction would have significantly modified our results, as suggested by O'BRODOVICH and COATES [18]. Nevertheless, not doing so might have underestimated RC-DTPA and increased its variability.

The extent of inflammation has been assessed by open lung biopsy in only 10 patients, and BAL was the only available way to estimate the inflammation within the lung parenchyma. Because inflammation of the alveolar walls and parenchyma may be reflected by intra-alveolar accumulation of cells and leaking of injured endothelium and epithelium into alveolar lining fluid, it has been advocated that an air space lavage specimen may capture these changes [3, 11, 13, 14, 29, 30]. BAL reflects regional intraluminal alveolitis. This gives an instantaneous picture of alveolitis but does not take into account its duration, which may be an important factor in the development of respiratory membrane damage. Indeed, HASLAM *et al.* [30] did not succeed in finding a relationship between semiquantitative scores of cell types observed within alveolar spaces and in alveolar walls and cell counts obtained from biopsy extractions or BAL in patients with DFA. However, two studies suggest that BAL provides relevant information on pulmonary inflammation. HUNNINGHAKE *et al.* [12] reported a close correlation between the proportions of various inflammatory and immune effector cells isolated from the lung of normal subjects, as well as from biopsies of patients with DFA, and those cell populations that were present in respective BAL fluids. WATTERS *et al.* [15] found that in patients with DFA, BAL lymphocytosis is associated with alveolar septal inflammation on the open lung biopsy.

RC-DTPA was increased in 25 of our 30 patients with DFA. Several studies have shown an increase in RC-DTPA in nonsmokers with DFA. CHOPRA *et al.* [22] noted that RC-DTPA was faster in the lower regions of the lungs of subjects suffering from pulmonary manifestations of scleroderma. RINDERKNECHT *et al.* [20] measured RC-DTPA in subjects with chronic interstitial lung diseases of various origins, and found it increased in five patients with Hamman and Rich pulmonary fibrosis. DUSSER *et al.* [23] reported that RC-DTPA was increased in seven patients with DFA. HARRISON *et al.* [31] investigated potential pulmonary involvement in patients with systemic sclerosis. They assessed thin section computed tomographic (CT) scan, BAL and RC-DTPA in defining pulmonary abnormalities in patients with normal chest radiograms: RC-DTPA was faster, compared to healthy control subjects, in 10 out of 14 patients with an abnormal chest radiogram and 7 out of 15 patients with a normal chest radiogram.

In our study, RC-DTPA correlated with the BAL lymphocyte counts; and, as a whole, in patients who received prednisolone, the initially increased RC-DTPA values decreased as VC did not deteriorate, and even improved in 6 out of 14. RC-DTPA does correlate with alveolar lymphocytosis in various conditions. MEIGNAN *et al.* [32] reported that RC-DTPA is increased in human immunodeficiency virus (HIV)-related cytotoxic lymphocytic

alveolitis in the absence of any obvious lung infection or tumor. HERVÉ *et al.* [33] demonstrated that in lung transplant patients during transplant rejection, RC-DTPA was increased and was associated with an increased proportion of lymphocytes in BAL, and returned to prerejection values with augmented immunosuppression.

Because impairment in lung volumes in DFA is induced by fibrotic lesions and by cellular infiltration of lung parenchyma, and because fibrotic lesions are not reversible, both the decrease in RC-DTPA and the lack of further impairment in VC during corticosteroid therapy were probably due to the anti-inflammatory action of prednisolone. The link between individual changes in VC during prednisolone therapy and the initial RC-DTPA could be explained by the pulmonary inflammation, which may account for the decrease in VC, the increase in RC-DTPA and its diminution under the action of prednisolone. Such an interpretation is supported by two studies in patients with DFA. RUDD *et al.* [4] showed that pulmonary function frequently improved with prednisolone in patients who initially had increased in lymphocytes in BAL fluid, but rarely in patients with increased neutrophils without lymphocytes. WATTERS *et al.* [15] observed that pretreatment lymphocytosis was associated with significant subsequent clinical improvement in response to corticosteroid therapy. Our study indicates that the VC of patients with initial slightly to moderately increased clearance rates will worsen, whilst that of patients with initial severely "leaky" lung will significantly improve under corticosteroid therapy (fig. 4). In addition, all patients in this study in whom DTPA clearance rates decreased under corticosteroid therapy had initially increased lymphocyte counts. However, RC-DTPA remained abnormal during therapy with prednisolone, indicating either an incomplete control of inflammation by corticosteroid or other factors responsible for the increase in RC-DTPA. In fact, alveolar lymphocytosis accounts for only 20% of the total variation in RC-DTPA. Other factors could be changes in surfactant and/or thickness of the hypophase, and stretching of the respiratory epithelium.

RC-DTPA correlated negatively with VC, which explains 21% of the total variation in RC-DTPA. Thus, we believe that the increased RC-DTPA in DFA could, in part, be explained by the fibrosis-induced increased recoil of the lung, which causes stretching of the respiratory epithelium similar to that caused by the increase in the lung volume. Several studies have shown that increasing lung volume augments RC-DTPA [20–22, 34]. This might be explained by stretching of intercellular junctions through which ^{99m}Tc -DTPA crosses the respiratory epithelium, and which renders the respiratory membrane more permeable to solutes [17]. Mechanical stretching of the respiratory epithelium probably occurs in DFA, where the decrease in VC appears to correlate closely with the degree of lung fibrosis and lung recoil [35–37].

Given the interdependence between normal and fibrotic units within the lung, even the expansion of structurally normal units might be affected [37]. Stretching of the respiratory epithelium may open the intercellular junctions through which DTPA is likely to cross the respiratory epithelium. The stretching, which is not reversible, may

account for some of the persistently increased RC-DTPA in patients who received prednisolone.

In conclusion, the RC-DTPA increase in patients with DFA could be related to the opening of intercellular junctions induced both by inflammation and increased recoil. Corticosteroid therapy might be responsible for the decrease - not a return to normal range - in RC-DTPA. This phenomenon might be explained by a decrease in inflammation and not in recoil, which is related to the persistent pulmonary fibrosis.

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