Imaging of pulmonary disease in scleroderma with J001X scintigraphy

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Abstract

Background – J001X is an acetylated polygalactoside isolated from the membrane of Klebsiella and able to interact with macrophages, mainly in their activated state. The aim of the present study was to determine the ability of 99m-labelled technetium (99mTc)-J001X scintigraphy to image pulmonary disease, defined by high resolution computed tomographic (HRCT) scanning and bronchoalveolar lavage (BAL) fluid analysis. Several techniques, including the total pulmonary diffusing capacity of carbon monoxide (TLCO), 99mTc-DTPA scintigraphy, 7 gallium-67 scintigraphy 10 and, more importantly, thin section high resolution computed tomographic (HRCT) scanning and bronchoalveolar lavage (BAL) fluid analysis 11, 12 have proved sensitive for detecting pulmonary disease in patients with scleroderma. Neutrophil or lymphocytic alveolitis, or both, associated with moderate eosinophilia are the most common abnormalities detected in BAL fluid from these patients. 12 However, alveolar macrophages are involved in the fibroin process through the secretion of interleukin 1, fibronectin, macrophage derived growth factor, and superoxide anion. 13 These activated cells are recruited to the lung by lymphocytes and neutrophils 14 and in vivo macrophage targeting appears to be a potential strategy for the scintigraphic imaging of such cases.

J001X, an acetylated polygalactoside isolated from the membrane of a non-pathogenic and non-encapsulated strain of Klebsiella pneumoniae, was developed to target macrophages selectively. 17 This molecule is able to interact with macrophages, mainly in their activated state, and in vitro studies have shown that CD11b/CD18 and CD14 molecules are involved in cell-J001X interaction. 18 In a recent unpublished study (P Haslam, personal communication) binding to ex vivo human alveolar macrophages using biotinylated J001X and the streptavidin-phycocerythrin amplification system in flow cytometry was described. The study was performed at three different concentrations of J001X in the presence or absence of an excess of unlabelled J001X in different models of pulmonary inflammation. The results confirmed that pulmonary inflammatory diseases are characterised by a recruitment of J001X binding macrophages, but without increased expression of J001X receptors. The binding was concentration dependent and totally displaced by unlabelled J001X. In vivo 99mTc-J001X scintigraphy has proved to be useful for imaging mediastinal lymph nodes and pulmonary disease caused by experimental chronic berylliosis in baboons 19 and sarcoidosis in man. 20

The aim of the present study was to determine the ability of J001X scintigraphy to image pulmonary disease, defined according to HRCT scanning, TLCO, and BAL fluid analysis, in patients suffering from scleroderma.

Scleroderma is a systemic disease characterised by an excess of collagen in the skin 1 and other organs. 2 Pulmonary involvement, assessed histologically, can be detected in 70% 3 to 100% 6 of patients. The mechanisms underlying pulmonary sclerosis seem to be common to scleroderma and to fibrosis from other causes; alveolar and interstitial injury are followed by collagen infiltration. 5 Clinical and pathological manifestations appear some time after the beginning of the disease. 6, 8 Pulmonary involvement should be detected early with the aim of protecting patients from tobacco and other environmental contaminants and to consider immunosuppressive treatment. 6

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Methods

STUDY POPULATION
Seventeen patients (16 women) aged 23–81 years suffering from scleroderma, with or without clinical respiratory symptoms, admitted consecutively to our institution between October 1990 and February 1991 were included in the study. Scleroderma was defined according to the American Rheumatism Association criteria.22 All patients gave written informed consent and the study was approved by the institution ethics committee. Thirteen patients were non-smokers and two had stopped one and three years previously. Two patients were current smokers (eight and 20 pack years). None had a recent past history or signs of respiratory infection at the time of the study. All had normal renal function and two had chronic hepatitis. The time from diagnosis to beginning the study ranged from one month to 37 years with a mean of 12 years. All patients had Raynaud’s phenomenon; seven had CREST syndrome23 and three Sjögren’s syndrome.24 The extent of cutaneous disease was classified in two ways25: acrosclerosis (seven patients) and proximal ascending scleroderma (10 patients). No patient had truncal scleroderma. Treatment included corticosteroids (one patient), non-steroidal anti-inflammatory drugs (one patient), calcium channel blockers (10 patients), factor XIII (three patients), parenteral prostacyclin (two patients), colchicine (two patients), sulphasalazine (one patient), and d-penicillamine (one patient).

A previous study including a control group had been performed in patients with sarcoidosis.21 In the control group we showed that lung clearance was always complete five hours after J001X inhalation; neither localised nor diffuse activity was observed in the thorax at five hours. In view of this previous experience a control group was not included in the present study.

COMPUTED TOMOGRAPHIC SCANNING
High resolution computed tomographic (HRCT) scans were carried out with a Somatom HIQ CT scanner at an exposure time of two seconds. Two millimetre thick slices performed at the end of inspiration were obtained at intervals of 10 mm on a 512 × 512 matrix from the apices to the bases. Prone sections were obtained when false positive results due to gravity dependent perfusion were suspected on supine sections.

HRCT scans were read by two independent radiologists blind to the results of other diagnostic studies. To assess interstitial disease the presence of micronodules, ground glass, septal thickening, subpleural linear opacities, and honeycombing appearance were noted.

PULMONARY FUNCTION TESTS
Forced inspiratory and expiratory flow volume curves and absolute lung volumes were measured in a constant pressure plethysmograph (Sensor Medics 2800, USA). A 10 second single breath carbon monoxide transfer test (TLCO, Morgan, UK) was measured on the day of each scintigraphic scan. Results were expressed as percentages of normal values.26 27 Expiratory flow rates, total lung capacity, and TLCO were considered to be decreased if they were less than 15% of normal values.

J001X SCINTIGRAPHY
One mg freeze dried J001X (Laboratoire Pierre Fabre, France) was labelled with 1100 MBq sodium 125I pertechnetate reduced in the presence of 125 µg stannous fluoride (Hepatate II, Amersham, UK). Patients inhaled the 4 ml radiolabelled J001X preparation as an aerosol in a specially devised aspiration hood (ESI, France) using an ultrasound TV 6000 inhaler (Siemens, West Germany) operating at 110 kHz as previously described.21 Three and five hours after inhalation, anterior and posterior 128 × 128 pixel images were recorded with a LFOV gamma camera (Siemens, Germany) equipped with a high resolution, low energy parallel collimator and connected to a S2000 computer for image processing (Sopha Medical, France). Each of the acquisition (anterior and posterior views) recorded three and five hours after inhalation was interpreted independently by two observers (PD and JLB). Background was not subtracted. Contrast was digitally enhanced by scaling the maximum intensity to a lower value than the maximum count. Positivity of J001X scintigraphy was defined by symmetrical bilateral pulmonary fixation three and five hours after inhalation of 125I-J001X. According to our previous experience of J001X scintigraphy in control subjects,21 125I-Tc-J001X activity localised in the oesophagus, stomach, heart, and breast was considered normal. Negative J001X scintigraphic findings were characterised by the absence of any thoracic activity at five hours as previously described.20

BRONCHOALVEOLAR LAVAGE (BAL)
Bronchoalveolar lavage was performed within 10 days of J001X scintigraphy according to the recommendations of the European Respiratory Society.28 Briefly, 150 ml sterile saline were infused in 20 ml aliquots at room temperature to the lingula or the middle lobe and immediately gently reaspirated. The first aliquot was collected separately and not used for analysis. Percentage of recovery was noted. After centrifugation, lavaged fluid cells were analysed for total numbers and differential counts. After staining with modified Wright-Giemsa stain, a minimum of 300 cells was counted to obtain a differential cell count. According to our own laboratory controls lymphocytes in BAL were considered to be increased when they constituted more than 20% of the total cells recovered.

DATA ANALYSIS
Patients were considered to have pulmonary scleroderma when they had at least two posi-
Table 1  Results in 12 patients with abnormal HRCT scans and pulmonary function tests

<table>
<thead>
<tr>
<th>Patient</th>
<th>PFT</th>
<th>HRCT</th>
<th>BAL</th>
<th>300X</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Restrictive syndrome Decrease in TLCO</td>
<td>Bilateral interstitial lung disease</td>
<td>L 75%, N 4%</td>
<td>BLF</td>
</tr>
<tr>
<td>2</td>
<td>Obstructive syndrome Decrease in TLCO</td>
<td>Left upper lobe interstitial disease</td>
<td>L 38%, N 5%</td>
<td>BLF</td>
</tr>
<tr>
<td>3</td>
<td>Restrictive syndrome Decrease in TLCO</td>
<td>Mild right lung interstitial disease</td>
<td>L 23%, N 4%</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>Restrictive syndrome Decrease in TLCO</td>
<td>Bilateral interstitial lung disease</td>
<td>L 1%, N 1%</td>
<td>BLF</td>
</tr>
<tr>
<td>5</td>
<td>Normal</td>
<td>Bilateral interstitial lung disease</td>
<td>L 1%, N 0%</td>
<td>BLF</td>
</tr>
<tr>
<td>6</td>
<td>Obstructive syndrome</td>
<td>Mild bilateral interstitial lung disease</td>
<td>L 2%, N 0%</td>
<td>BLF</td>
</tr>
<tr>
<td>7</td>
<td>Restrictive syndrome Decrease in TLCO</td>
<td>Centrilobular emphysema</td>
<td>L 3%, N 1%</td>
<td>BLF</td>
</tr>
<tr>
<td>8</td>
<td>Restrictive syndrome Decrease in TLCO</td>
<td>Left upper lobe interstitial lung disease</td>
<td>L 10%, N 5%</td>
<td>BLF</td>
</tr>
<tr>
<td>9</td>
<td>Normal</td>
<td>Mild bilateral interstitial lung disease</td>
<td>L 1%, N 0%</td>
<td>BLF</td>
</tr>
<tr>
<td>10</td>
<td>Obstructive syndrome Decrease in TLCO</td>
<td>Bilateral upper lobe interstitial lung disease</td>
<td>L 4%, N 1%</td>
<td>Negative</td>
</tr>
<tr>
<td>11</td>
<td>Restrictive syndrome Decrease in TLCO</td>
<td>Not available</td>
<td>Not available</td>
<td>BLF</td>
</tr>
<tr>
<td>12</td>
<td>Obstructive syndrome</td>
<td>Bilateral upper lobe interstitial lung disease</td>
<td>Not available</td>
<td>BLF</td>
</tr>
</tbody>
</table>

PFT = pulmonary function tests; BAL = bronchoalveolar lavage; L = lymphocytes; HRCT = high resolution computed tomography; N = neutrophils; TLCO = carbon monoxide transfer factor.

Table 2  Results in 5 patients with normal pulmonary function tests and HRCT scans

<table>
<thead>
<tr>
<th>Patient</th>
<th>BAL</th>
<th>300X</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Bilateral lung fixation</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Bilateral lung fixation</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

BAL = bronchoalveolar lavage; L = lymphocytes; N = neutrophils; HRCT = high resolution computed tomography.

had more than 20% lymphocytes in the BAL fluid.

Thus, 12 of 17 patients had positive J001X scintigraphic results. Ten of these patients were considered to have pulmonary scleroderma with abnormal pulmonary function tests or HRCT scans, or both, and two were considered to have no signs of pulmonary scleroderma. There was no relation between BAL lymphocytosis and J001X scintigraphy results, or between BAL lymphocytosis and pulmonary scleroderma: four patients had more than 20% lymphocytes in the BAL fluid and two of these had positive J001X scintigraphic results.

Discussion

In this study in patients with scleroderma J001X scintigraphic scans were positive in 10 of the 12 patients suspected of having pulmonary disease and in two of the five patients without pulmonary disease as defined by HRCT scanning and lung function.

The "gold standard" for assessing pulmonary involvement in scleroderma is still considered to be open lung biopsy. Nevertheless, a recent study has suggested that HRCT scanning may be as good, and it has the advantage of imaging the entire lungs. 

For ethical reasons patients included in this study who were all clinically asymptomatic were not submitted to a biopsy. HRCT scanning and pulmonary function tests, including TLCO, which are sensitive tests of pulmonary involvement in patients suffering from scleroderma, were chosen as reference tests. Nevertheless, the specificity of some signs on HRCT scans, such as septal thickening or subpleural linear opacities, is controversial when they occur in isolation. This is why we considered that at least two signs of interstitial lung disease were necessary apart from honeycombing which is specific to fibrosis. Scleroderma is known to induce vascular damage and depression in TLCO which is not specific for parenchymal disease. Patients were considered to have pulmonary scleroderma when a decrease in TLCO was associated with reduced lung volumes and two positive signs on HRCT scanning without any event other than scleroderma in their medical history.

J001X is a fully characterised 34 kDa acylated poly(1,3)galactoside isolated from Klebsiella membranes which is able to bind selectively to recruited macrophages. Because of its amphipathic properties 99Tc-J001X is

**Note:** The text contains references to medical and scientific terminology and conditions, which are not explicitly expanded in the provided text. The provided text is a representation of the document in question, focused on translating it into a natural text format while maintaining the integrity of its informational content. This approach was intended to ensure that the natural text accurately reflects the original document's content, without introducing any new information or altering its contextually relevant details.
Imaging of pulmonary disease in scleroderma with 99mTc scintigraphy

Studies are now also in progress to define a quantitative or semiquantitative method for analysis of 99mTc scintigraphy based on the model of that used to process gallium scintigraphy. Only a prolonged follow up of a large population could provide an answer concerning the prognostic value of positive 99mTc scintigraphy and may provide a better knowledge of the process involving macrophages during pulmonary involvement of scleroderma.

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