Endothelial dysfunction in pulmonary arteries of patients with mild COPD

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Peinado, Víctor I., Joan A. Barberà, J osep Ramírez, Federico P. Gómez, Josep Roca, Lluís J over, Josep M. Gimferrer, and Robert Rodríguez-Roisin. Endothelial dysfunction in pulmonary arteries of patients with mild COPD. Am. J. Physiol. 274 (Lung Cell. Mol. Physiol. 18): L908–L913, 1998.—To investigate whether endothelial dysfunction of pulmonary arteries (PA) is present in patients with mild chronic obstructive pulmonary disease (COPD) and to what extent it is related to the morphological abnormalities of PA, we studied 41 patients who underwent lung resection. Patients were divided into the following groups: nonsmokers (n = 7), smokers with normal lung function (n = 13), and COPD (n = 21). Endothelium-dependent relaxation mediated by nitric oxide was evaluated in vitro in PA rings exposed to cumulative concentrations of acetylcholine (ACh) and ADP. Structural abnormalities of PA were assessed morphometrically. PA of COPD patients developed lower maximal relaxation in response to ADP than both nonsmokers and smokers (P < 0.05 each) and a trend to reduced relaxation in response to ACh (P = 0.08). Maximal relaxation to ADP correlated with the degree of airflow obstruction (r = 0.48, P < 0.01). Morphometrical analysis of PA revealed thicker intimas, especially in small arteries, in both smokers and COPD compared with nonsmokers (P < 0.05 each). We conclude that endothelial dysfunction of PA is already present in patients with mild COPD. In these patients, as well as in smokers with normal lung function, small arteries show thickened intimas, suggesting that tobacco consumption may play a critical role in the pathogenesis of pulmonary vascular abnormalities in COPD.

endothelium; intimal layer; nitric oxide; pulmonary circulation; tobacco smoking

ENDOTHELIAL-DERIVED nitric oxide (NO) is a potent vasodilator that plays an important role in modulating pulmonary vascular tone (14). Impairment of endothelium-dependent vascular relaxation has been shown in pulmonary arteries of patients with end-stage obstructive lung diseases (namely, bronchiectasis and emphysema) who underwent lung transplantation (15). In that study, the degree of impairment of vascular relaxation was correlated with both the severity of hypoxia and the thickening of the intimal layer of pulmonary arteries. Because hypoxia may attenuate the endothelium-dependent vascular relaxation (8), it was postulated that chronic hypoxemia might account for the diminished release of endothelium-derived relaxing factors (15). Moreover, reduced expression of NO synthase (NOS) has been shown in pulmonary arteries of patients with pulmonary hypertension, the immunoreactivity to NOS being inversely related to the structural derangement of pulmonary arteries (17). Accordingly, in pulmonary arteries of patients with advanced obstructive lung disorders with secondary pulmonary hypertension, the development of structural abnormalities may be accompanied by an impaired release of endothelium-derived NO, which in turn may further enhance the progression of pulmonary hypertension.

In a previous study, we showed that patients with mild chronic obstructive pulmonary disease (COPD) may exhibit intimal thickening in small pulmonary muscular arteries and that the degree of intimal thickening was associated with a reduced reactivity to hypoxic stimulus (4). Accordingly, we hypothesized that endothelial dysfunction might be already present in pulmonary arteries of patients with mild COPD. Because these patients usually do not exhibit severe hypoxemia, we postulated that, in mild COPD, the diminished release of endothelium-derived relaxing factors (namely, NO) could be related to the underlying structural derangement of pulmonary arteries.

The present study was therefore designed to assess in vitro the reactivity of pulmonary artery rings to NO-dependent and NO-independent vasodilators and to evaluate morphologically the abnormalities of pulmonary muscular arteries in patients with mild COPD and in control subjects who underwent lung resective surgery for lung carcinoma.

MATERIALS AND METHODS

Subjects. Forty-one patients (32 males and 9 females) who underwent lobectomy or pneumonectomy because of lung carcinoma were studied. Pulmonary function tests (forced spirometry, lung volumes, carbon monoxide diffusing capacity, and blood gas analysis) were performed in the days preceding surgery, as previously described (4).

On the basis of smoking history and the results of forced spirometry, patients were divided into the following three groups: 1) nonsmokers, all of whom had normal lung function; 2) smokers with normal lung function; and 3) COPD, smokers with airflow obstruction, as defined by a forced expiratory volume in 1s (FEV1)-to-forced vital capacity (FVC) ratio lower than 70%. General characteristics and lung function measurements of each group of patients are shown in Table 1.

In vitro assessment of vascular reactivity. Resected lung specimens were placed in cold Krebs-Henseleit buffer (in mM: 118 NaCl, 24 NaHCO3, 11.1 glucose, 4.7 KCl, 1.2 KH2PO4, 1.2 MgSO4, and 1.6 CaCl2) gassed with 95% O2-5% CO2 (pH = 7.35–7.45). Arterial segments with an external diameter of 1.5–2.5 mm were carefully dissected free of visible fat and...
ENDOTHELIAL DYSFUNCTION IN MILD COPD

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Results

Lung function measurements from the three groups are shown in Table 1. Patients in the COPD group showed moderate airflow obstruction (FEV₁, 72 ± 15% of predicted) and gas trapping compared with the other groups. COPD patients also showed lower arterial partial O₂ pressure (PaO₂) than nonsmokers, although it was >80 mmHg in the majority of patients.

Measurements of vascular reactivity. The results of reactivity studies in pulmonary artery rings from the three groups are summarized in Table 2. Maximal contraction to PE was similar in the three groups.
Table 2. Vascular responses of pulmonary artery rings

<table>
<thead>
<tr>
<th></th>
<th>Nonsmokers</th>
<th>Smokers</th>
<th>COPD</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contraction to PE (10^-5 M), mg</td>
<td>997±275</td>
<td>929±226</td>
<td>1,035±382</td>
<td>0.66</td>
</tr>
<tr>
<td>Maximal relaxation, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACh</td>
<td>40.1±22.0</td>
<td>41.1±19.8</td>
<td>24.9±22.9</td>
<td>0.08</td>
</tr>
<tr>
<td>ADP</td>
<td>81.0±14.3</td>
<td>73.4±14.1</td>
<td>56.0±22.0†</td>
<td>0.01</td>
</tr>
<tr>
<td>SNP</td>
<td>93.5±5.1</td>
<td>94.2±4.7</td>
<td>91.4±6.8</td>
<td>0.46</td>
</tr>
<tr>
<td>ACh+L-NAME</td>
<td>179±22.0</td>
<td>57.7±9.7</td>
<td>5.3±11.4</td>
<td>0.16</td>
</tr>
<tr>
<td>ADP+L-NAME</td>
<td>177±11.2</td>
<td>139±12.2</td>
<td>15.4±10.1</td>
<td>0.73</td>
</tr>
<tr>
<td>SNP+L-NAME</td>
<td>933±9.7</td>
<td>920±6.1</td>
<td>945±6.6</td>
<td>0.78</td>
</tr>
<tr>
<td>EC50—log[M]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACh</td>
<td>7.9±0.8</td>
<td>7.4±0.5</td>
<td>8.2±1.4</td>
<td>0.36</td>
</tr>
<tr>
<td>ADP</td>
<td>7.1±0.6</td>
<td>6.8±0.4</td>
<td>6.9±0.5</td>
<td>0.68</td>
</tr>
<tr>
<td>SNP</td>
<td>7.4±0.4</td>
<td>7.7±0.4</td>
<td>7.6±0.7</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Values are means ± SD. PE, L-phenylephrine; SNP, sodium nitroprusside; L-NAME, NG-monomethyl-L-arginine; EC50, concentration needed to reach 50% of maximal relaxation. P values determined using ANOVA. *P < 0.05 vs. nonsmokers. †P < 0.05 vs. smokers.

Compared with both nonsmokers and smokers, there was a significant reduction in the maximal relaxation in response to ADP in pulmonary artery rings in the COPD group (Table 2 and Fig. 1). Maximal relaxation to ACh was not significantly reduced in the mild COPD group (Table 2). However, the area under the dose-response curve to ACh, which summarizes the response at the different concentrations (Fig. 1), was greater in COPD than in nonsmokers (499±116 and 385±69, respectively, P < 0.05). No differences in the EC50 of either ACh or ADP dose-response studies were shown among the different groups. When artery rings were treated with SNP, all of the rings relaxed maximally, and no differences between groups were observed.

The decrease in vascular reactivity of arteries from COPD patients was not due to endothelial damage during manipulation, since the histological evaluation with factor VIII confirmed the presence of endothelial cells on the luminal side of the arterial wall. Moreover, when rings were exposed to the competitive inhibitor of NOS, L-NAME, relaxation induced by both ACh and ADP was almost abolished (Table 2), indicating that both vasodilators operated through the L-arginine-NO pathway.

The maximal relaxations induced by both ACh and ADP correlated with the FEV1-to-FVC ratio (r = 0.48, P < 0.01 and r = 0.38, P < 0.05, respectively; Fig. 2). Moreover, maximal relaxation induced by ADP was inversely correlated with the alveolar-arterial O2 pressure difference (r = −0.51, P < 0.01).

No differences in maximal relaxation to ACh and ADP were observed when arterial rings were incubated with and without L-arginine (P = 0.13 and P = 0.44, respectively), thus indicating that the diminished response to these agents was not due to a substrate deficiency.

Morphological evaluation. Morphometric measurements in pulmonary muscular arteries from the three groups of patients are shown in Table 3. The number of arteries analyzed in each subject was similar in the three groups. Likewise, there was a similar distribution in the external diameter and in the degree of narrowing of the arteries that were examined among the groups.

Both COPD patients and smokers showed thicker intimas than nonsmokers (Table 3). By contrast, no differences in the size of the muscular layer were shown. The degree of intimal thickening in COPD patients and smokers was similar.

Estimated parameters of the relationship between thickness of the intimal layer and calculated artery diameter disclosed differences between nonsmokers and both smokers and COPD (P = 0.033 and P = 0.003, respectively) that were significant in arteries with small diameters (<182 μm, nonsmokers vs. smokers; and <220 μm, nonsmokers vs. COPD). No differences...
between smokers and COPD were shown in the relationship between intimal thickness and artery diameter. Furthermore, the analysis of muscular size as a function of artery diameter showed no differences among the three groups.

**DISCUSSION**

The results of the present study show impaired relaxation to the NO-dependent vasodilator ADP in pulmonary arteries of patients with mild COPD, which was associated with enlargement of the intimal layer in small pulmonary muscular arteries. Moreover, in smokers with normal lung function, pulmonary muscular arteries also exhibited intimal thickening, although the NO-dependent relaxation did not differ from that in nonsmokers.

In our study, maximal relaxation of pulmonary artery rings induced by ADP was diminished in COPD patients. Relaxation induced by ACh was also diminished in the COPD group, as shown by a greater area under the dose-response curve, although its magnitude was lower than that shown with ADP (Table 2). This different response to ADP and to ACh might be related to the variable effects that ACh may exert on pulmonary arteries, where it may induce contraction when used at high concentrations (2). Contrasting with the response to NO-dependent vasodilators, relaxation induced by a direct NO donor, such as SNP, was maximal in the three study groups. Moreover, inhibition of NO synthesis with L-NAME practically abolished the response to NO-dependent vasodilators. Overall, these results are consistent with endothelial dysfunction in pulmonary arteries of patients with mild COPD.

Dinh-Xuan and co-workers (15) showed endothelial dysfunction in pulmonary arteries of patients with end-stage obstructive lung diseases (bronchiectasis and emphysema). Results of the present study are in agreement with this finding and extend it to the initial stage of the COPD spectrum. Accordingly, endothelial dysfunction of pulmonary arteries appears not to be a phenomenon restricted to advanced COPD. Yet, it might be initiated early on in the course of the disease when airflow obstruction is moderate and PaO₂ is within the normal range. Results of the present study also support our previous suggestion that the impairment of hypoxic pulmonary vasoconstriction in patients with mild COPD is more likely due to the functional impairment of endothelium-dependent relaxation than to the stiffness of the pulmonary artery wall produced by connective tissue proliferation. The correlation between maximal relaxation to both ACh and ADP and the severity of airflow obstruction (Fig. 2) suggest that endothelial dysfunction may be enhanced with disease progression. Interestingly, pulmonary arteries of smokers with normal lung function also showed a trend to lower relaxation in response to NO-dependent vasodilators (Table 2), although it was not significantly different from that in nonsmokers. This raises the possibility that endothelial impairment might originate even before airflow obstruction is apparent. In this regard, it is interesting to note that impairment of endothelium-dependent arterial dilatation of systemic arteries has been shown in both active and passive smokers (10, 24).

Dinh-Xuan and co-workers (15) showed that the degree of endothelium-dependent relaxation correlated with the PaO₂ value, suggesting that chronic hypoxia may impair endothelial-cell metabolism and the synthesis of endothelium-derived relaxing factors. Contrasting with this hypothesis, in our study, PaO₂ was within the normal range in the majority of COPD patients, the lowest individual value of PaO₂ in our series being 67 mmHg. Although some of our patients may show transitory episodes of hypoxemia worsening, i.e., at night or during exercise, it seems unlikely that hypoxemia by itself might have played a major pathogenetic role in the endothelial dysfunction of these patients. Accordingly, we consider that additional factors could be more relevant in altering endothelial function of pulmonary arteries at this early stage. Indeed, the effect of hypoxia on endothelial function of pulmonary arteries could be more relevant in altering endothelial function of pulmonary arteries at this early stage.

![Graph](image)

**Fig. 2.** Relationship between maximal relaxation in response to ACh, expressed as percent reduction from precontraction with PE, and the degree of airflow obstruction [forced expiratory volume in 1 s (FEV₁)-to-forced vital capacity (FVC) ratio] in all patients.

**Table 3.** Morphometric measurements on pulmonary muscular arteries

<table>
<thead>
<tr>
<th>Arteries/patient</th>
<th>Nonsmokers</th>
<th>Smokers</th>
<th>COPD</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured external diameter, µm</td>
<td>136 ± 70</td>
<td>133 ± 47</td>
<td>112 ± 22</td>
<td>0.27</td>
</tr>
<tr>
<td>Calculated external diameter, µm</td>
<td>242 ± 139</td>
<td>229 ± 67</td>
<td>200 ± 37</td>
<td>0.32</td>
</tr>
<tr>
<td>Index of narrowing, %</td>
<td>54 ± 5</td>
<td>56 ± 7</td>
<td>55 ± 8</td>
<td>0.79</td>
</tr>
<tr>
<td>Total area, mm² 10⁻⁴</td>
<td>291 ± 299</td>
<td>244 ± 151</td>
<td>184 ± 94</td>
<td>0.28</td>
</tr>
<tr>
<td>Wall thickness, % measured radius</td>
<td>40.8 ± 7.9</td>
<td>47.9 ± 9.0</td>
<td>48.7 ± 8.1</td>
<td>0.10</td>
</tr>
<tr>
<td>Lumen area, % total area</td>
<td>43.3 ± 7.7</td>
<td>36.6 ± 9.3</td>
<td>35.4 ± 7.9</td>
<td>0.10</td>
</tr>
<tr>
<td>Intimal area, % total area</td>
<td>20.9 ± 4.9</td>
<td>27.1 ± 5.6*</td>
<td>28.2 ± 6.0*</td>
<td>0.02</td>
</tr>
<tr>
<td>Muscular area, % total area</td>
<td>35.7 ± 4.5</td>
<td>36.3 ± 7.1</td>
<td>36.5 ± 6.8</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Values are means ± SD. P values determined using ANOVA. Index of narrowing determined by equation 100·[1−(measured total area/calculted total area)]. *P < 0.05 vs. nonsmokers.
is controversial. Whereas some studies have shown that hypoxia may downregulate NO synthesis (8), others seem to indicate that it may exert the opposite effect (7, 21, 31).

Results of morphometric measurements of pulmonary muscular arteries in the present series are in agreement with data previously reported by our group (4). Compared with nonsmokers, patients with mild COPD showed thickening of the intimal layer of small pulmonary muscular arteries (Table 3). This finding reinforces the notion that structural alterations of the intimal layer in small pulmonary muscular arteries may be an early feature in COPD. Furthermore, in the present study, we have shown intimal thickening in pulmonary arteries of smokers who had normal lung function to a similar degree to that seen in COPD patients (Table 3). This suggests that tobacco consumption might be associated with structural abnormalities of pulmonary vessels, even when airflow obstruction is not apparent. Accordingly, tobacco consumption seems to play a pivotal role in the remodeling process of pulmonary vasculature. MacNee and associates (22) showed that active cigarette smoking delays the transit of neutrophils in the pulmonary circulation and increases their capability to interact with pulmonary endothelium. Indeed, activation of endothelium by inflammatory mediators in pulmonary vessels of smokers has been shown by an increased expression of inducible adhesion molecules (E- and P-selectin and intercellular adhesion molecule-1; see Ref. 18). Endothelial activation may promote the release of cytokines and growth factors (13, 29) that induce the proliferation of fibroblasts and the laying down of cell matrix proteins (9).

Different studies have related the morphological abnormalities of the vessel wall with an impaired release of NO by endothelial cells. Giaid and Saleh (17) demonstrated that, in patients with primary pulmonary hypertension, endothelial NOS (eNOS) immunoreactivity was reduced in those cases in which more severe grades of arteriopathy were exhibited. Furthermore, Dinh-Xuan and colleagues (15) also showed in patients with end-stage COPD an inverse relationship between thickening of the intimal layer and the relaxation induced by ACh. In our study, pulmonary arteries in the COPD group were those that showed the lowest reactivity to NO-dependent vasodilators and the greatest thickening of the intimal layer (Fig. 3). To what extent the diminished release of NO by endothelial cells contributes to the intimal enlargement or is the consequence of a persistent structural damage remains uncertain. NO possesses antiproliferative effects that may suppress both neointimal vascular thickening and angiogenesis (23, 25). In addition, NO inhibition has been shown to increase collagenous protein synthesis (25). However, results of our study show intimal thickening in pulmonary arteries of smokers whose endothelial function did not differ significantly from that of nonsmokers (Table 3 and Fig. 3), suggesting that structural changes might antecede or be independent of the impairment of pulmonary vascular reactivity. Cigarette smoke induces cell proliferation in small vessels (27) and alters endothelium permeability (1), changes that may ensue either from a direct effect of cigarette smoke on pulmonary vasculature or from the release of cytokines by inflammatory cells, which are commonly present in the airways of chronic smokers (3, 5, 12). Because the severity of bronchial inflammation correlates with that of pulmonary vessel abnormalities (4, 19, 30), we hypothesize that the inflammatory process associated with tobacco consumption may play a key role in the pathogenesis of pulmonary vascular abnormalities of COPD. In this scenario, the continuous release of NO by endothelial cells may be relevant in preserving the integrity of the vessel wall under both physiological and pathological conditions, since endothelium-derived NO not only modulates the vascular tone but also regulates the interactions between vascular endothelium and circulating inflammatory cells (11, 20, 26). The latter might be particularly relevant at sites of inflammation, where endothelium must protect itself against toxic mediators released from chemoattractant-stimulated, emigrating neutrophils. If this is the case, pulmonary vascular remodeling in COPD may follow, at least in part, a pathogenesis similar to that proposed in other pulmonary hypertensive diseases (29). Accordingly, it can be hypothesized that the inflammatory process associated with tobacco smoking might be responsible, on the one hand, for the enlargement of the intimal layer through the release of growth factors and, on the other, for the reduced expression of eNOS as a consequence of the action of inflammatory mediators. Reduced NO release can diminish vascular reactivity and, additionally, may further promote and perpetuate the remodeling process of the vascular wall, since NO also possesses antiproliferative properties (28, 32). Further studies characterizing the inflammatory infiltrate and the expression of eNOS in pulmonary arteries of both smokers and patients with mild COPD should contribute to elucidate this hypothesis.

In summary, the present study shows that endothelial dysfunction of pulmonary arteries may be already present in patients with mild COPD. Furthermore, the
observation that smokers with normal lung function exhibited intimal thickening in small pulmonary arteries suggests that tobacco consumption may play a central role in the pathogenesis of the structural and functional alterations of the pulmonary vasculature in COPD.

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