Nitric oxide and carbon monoxide lung transfer in patients with advanced liver cirrhosis

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Submitted 19 December 2008; accepted in final form 7 May 2009

Nitric oxide and carbon monoxide lung transfer in patients with advanced liver cirrhosis. J Appl Physiol 107: 139–143, 2009. First published May 7, 2009; doi:10.1152/japplphysiol.91621.2008.—In advanced cirrhosis, decreased lung transfer for carbon monoxide (TLCO) and increased alveolar-arterial oxygen tension difference (PA-aO2 ≥15 mmHg while breathing ambient air) are frequently detected. Pulmonary membrane diffusion capacity for CO (DmCO) and pulmonary capillary blood volume (Vcap) can be derived from the simultaneous measurement of TLCO and lung transfer for nitric oxide (TLNO). Measurements of single-breath TLNO and TLCO were performed in 49 cirrhotic patients with advanced liver cirrhosis and in 35 healthy controls to derive Vcap, DmCO, and TLNO:TLCO ratio. Twenty-five patients had increased PA-aO2 of whom 11 had hepatopulmonary syndrome (HPS). Compared with controls, non-HPS patients with normal PA-aO2 had a significant ∼10% decrease in TLCO, DmCO, and Vcap but similar TLNO:TLCO ratios. Compared with non-HPS patients with normal PA-aO2, non-HPS patients with increased PA-aO2 had lower Vcap and higher TLNO:TLCO ratio but similar DmCO. HPS patients had lower Vcap and higher TLNO:TLCO ratios than both subgroups of non-HPS patients. In cirrhotic patients, TLNO:TLCO ratios correlated positively, and TLCO (percentage of the predicted value) and Vcap (percentage of the predicted value) correlated negatively with PA-aO2 (r2 = 0.25, P = 0.0003; r2 = 0.48, P < 0.0001 and r2 = 0.57, P < 0.0001, respectively). We concluded that, in cirrhotic patients, lower TLCO and increased PA-aO2 are associated with lower Vcap. In addition, high TLNO:TLCO ratios in patients with increased PA-aO2 suggest a decreased thickness of the capillary blood layer in these patients.

capillary blood volume

LUNG TRANSFER FOR CARBON MONOXIDE (TLCO) is a valuable tool in assessing pulmonary disease (10). Reduction in TLCO is frequently noted in patients with advanced liver disease (14). Decreased lung compliance caused by massive hepatomegaly, ascites, or pleural effusions, or by a recognizable pulmonary or cardiac disorder, can account for this abnormality (9, 17). Reduced TLCO also appears to be a common functional marker of hepatopulmonary syndrome (HPS), a complication of hepatic disease that consists in abnormal dilatation of pulmonary precapillary and capillary vessels either with or without pulmonary arteriovenous communications, whereas alveolar ventilation is preserved (20). In addition, a subset of cirrhotic patients without HPS or other identified pulmonary or cardiac disorders also have reduced TLCO (12).

The process of pulmonary CO uptake can be simplified into two transfers or conductances, membrane conductance for CO (DmCO), which reflects the diffusion properties of the alveolar capillary membrane, and conductance of CO on hemoglobin (Hb), which is the product of the CO-Hb chemical reaction rate (90C) and the mass of Hb in the alveolar capillary blood volume (Vcap). Because these two conductances are in series, they are related by the equation proposed by Roughton and Forster [1/TLCO = (1/DmCO) + (1/(6C × Vcap)))] (23). The solution to this equation with two unknowns, DmCO and Vcap, can be obtained by simultaneously measuring TLCO and lung transfer for nitric oxide (TLNO) (8). A decrease in DmCO is interpreted as a “thickening” of the alveolo-capillary membrane or as a decrease in lung area and a decrease of Vcap as decreased blood volume in the ventilated alveoli. The equation of Roughton and Forster assumes that DmCO and Vcap are independent components (23). However, the surface areas of the alveolar membrane and of the capillaries are identical or are closely related; consequently, DmCO and Vcap should be directly correlated (7, 24). Under this assumption, Glenet et al. (7) recently demonstrated that the TLNO:TLCO ratio is independent of the membrane surface area and inversely proportional to the thicknesses of the alveolar membrane and of the capillary blood layer. The mechanisms of low TLCO remain unresolved in patients with advanced liver disease (21). Therefore, to analyze the lung diffusion properties of these patients, we simultaneously measured TLCO and TLNO in 49 consecutive patients who had no primary lung and/or cardiac disease and who were awaiting liver transplantation.

MATERIALS AND METHODS

Patients. From September 2007 to October 2008, 49 consecutive patients on the liver-transplantation waiting list at Toulouse University Hospital and 35 healthy control subjects matched with patients for age, height, and weight were investigated. All patients had biopsy-proven liver cirrhosis. Physical examination findings and blood data were analyzed to determine severity of liver disease according to modified Child-Pugh criteria (grade A vs. B vs. C) (19). Patients with primary lung disease, as assessed by medical history, physical examination, chest X-ray, and pulmonary function tests, were not included in this study. In particular, patients with any stage of interstitial lung disease were excluded. Patients were also excluded if they had cardiac disease (including systolic and diastolic left heart disease, mitral and/or aortic stenosis and/or regurgitation, and pulmonary arterial hypertension) as assessed by medical history, physical examination, Doppler echocardiography, and/or right heart catheterization. The

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study was approved by the Toulouse University Hospital Institutional Review Board, and informed consent was obtained from each patient.

**Pulmonary function.** Spirometry was performed according to the European Respiratory Society guidelines (27).

Arterial blood gases were analyzed in patients breathing room air in a sitting position. Alveolar-arterial oxygen tension differences (PA-aO2) were calculated as described elsewhere (21).

In cirrhotic patients, diagnosis of HPS was based on the presence of oxygenation defect [arterial hypoxemia (arterial partial pressure of oxygen <80 mmHg) and/or increased PA-aO2 i.e., ≥15 mmHg) while breathing ambient air], together with positive contrast-enhanced echocardiography, in accordance with present recommendations (20).

**Measurement of TLCO and TLNO.** TLNO and TLCO were measured simultaneously during a single breath maneuver using an automated apparatus (Medisoft, Dinant, Belgium), as described elsewhere (1). Briefly, subjects in a sitting position breathed through a mouthpiece while wearing a nose clip. The air mix contained 0.28% CO, 14% He, and 21% O2 balanced with N2, which was mixed with a NO2 mixture (450 ppm NO2; Air Liquide, Sainte, France). The final concentration of NO in the inspired bag was 40 ppm, and that of O2 was 19.1%. A breath hold of 4 s was requested followed by rapid expiration. The first 0.9 l of expired gas was rejected, and a further 0.9 l was automatically analyzed for NO, CO, and He. The total breath-holding time was calculated from the beginning of inspiration (minus 30% of the inspiratory time) to the middle of the expired gas sample (10). The alveolar volume (VA) during apneaus was estimated using the helium-dilution technique. TLCO was corrected for Hb concentration and for CO Hb concentration in all subjects (10). Values of DmCO and Vcap were calculated according to the equation of Roughton and Forster (23):

\[
1/\text{TLCO} = (1/\text{DmCO}) + [1/(\theta \text{CO} \times \text{Vcap})]
\]

where θCO reference values were from Forster, as previously described (1). Equation 1 was applied to lung transfer for NO; 1/θNO was assumed to be negligible, and then

\[
\text{TLNO} = \text{DmNO} = a \text{DmCO}
\]

where a = 1.97 following Graham’s law (15). Equations 1 and 2 give

\[
\text{TLNO:TLCO} = a [1 + \text{DmCO}(\theta \text{CO} \times \text{Vcap})]
\]

The surface area of the alveolar membrane and of the capillaries are identical or closely related (7). Consequently, a change in alveolo-capillary membrane area will affect DmCO and Vcap in the same way, and, from Eq. 3, the TLNO:TLCO ratio will be unchanged. An increase of the TLNO:TLCO ratio then implies either a decrease in the thickness of the alveolar membrane (increasing DmCO) and/or a decrease in the “thicknes component” of Vcap. By contrast, a decrease of the TLNO:TLCO ratio implies either an increase in the thickness of the alveolar membrane (increasing DmCO) and/or an increase in the “thickness component” of Vcap.

As age, height, and sex independently influence DmCO and Vcap, a percentage of the predicted value was considered for interindividual comparisons. The reference values for DmCO and Vcap were from Aguilaniu et al. (1).

**Statistical analyses.** Data were expressed as means ± SD. For between-group comparisons, ANOVA was performed. For correlation analysis, Pearson’s correlation coefficient (r) and a simple linear regression using the least-squares method was used. A P value <0.05 was considered significant. Analyses were performed using StatView, version 5.0 (SAS Institute, Cary, NC).

**RESULTS**

During the study period (14 mo), 62 consecutive patients were evaluated. Three patients were unable to perform the test satisfactorily and were not included. Eight additional patients were excluded because of primary lung disease (interstitial lung disease in two cases, chronic obstructive pulmonary disease in six cases), and a further two patients were excluded because of portopulmonary hypertension.

Physical and clinical characteristics of patients and controls are shown in Table 1. Of the 49 cirrhotic patients, 11 met the criteria for HPS. Patients and controls were similar in age, height, weight, and smoking habits. Cirrhotic patients with and without HPS (non-HPS) had a similar distribution for etiology and Child-Pugh grade. However, dyspnea was more prevalent in HPS patients than in non-HPS patients.

Blood gas analysis and lung function measurements of patients and controls are shown in Table 2. Arterial hypoxemia was present in 2/38 cirrhotic non-HPS patients and in 9/11 HPS patients. Increased PA-aO2 was present in 25 patients including 14 non-HPS patients. Spirometric values did not differ between healthy subjects and cirrhotic patients. VA did not significantly differ between groups. Moreover, VA expressed as a percentage of TLC was close to 100% in patients and controls, indicating that a full inspiration was performed during the single-breath transfer maneuver.

In cirrhotic patients, we found no association between reduced TLCO and present or former tobacco use (data not shown). Non-HPS patients with normal PA-aO2 had a moderate but significant decrease (~10%) in TLCO, Vcap, and DmCO but similar TLNO:TLCO ratio compared with healthy controls. Non-HPS patients with increased PA-aO2 had higher TLNO:TLCO ratios and lower Vcap (percentage of the predicted value) compared with non-HPS patients with normal PA-aO2 (4.53 ± 0.37 vs. 4.26 ± 0.44, P = 0.001, and 81 ± 14% vs. 93 ± 15%, P < 0.0001, respectively). Compared with all subgroups of patients, HPS patients had lower TLCO, lower Vcap, and higher TLNO:TLCO ratios.

There was a strong negative correlation between TLCO and PA-aO2 (r2 = 0.48, P < 0.0001; Fig. 1A) and between Vcap and PA-aO2 (r2 = 0.57, P < 0.0001; Fig. 1B). By contrast, there was a weak but significant positive correlation between TLNO:TLCO ratio and PA-aO2 (r2 = 0.25, P = 0.0003; Fig. 1C).

**DISCUSSION**

Impaired TLCO is a common finding in patients with advanced cirrhosis, but the mechanisms of this abnormality are unclear. It has been suggested that arterial oxygenation abnor-

| Table 1. Physical and clinical characteristics of healthy subjects (controls) and cirrhotic patients either with or without HPS |
|-----------------|-----------------|-----------------|
|                 | Controls (n = 35) | non-HPS (n = 38) | HPS (n = 11) |
| Sex, women/men  | 8/27            | 10/28           | 3/9           |
| Age, yr         | 52 ± 10         | 52 ± 12         | 54 ± 7        |
| Height, m       | 169 ± 8         | 168 ± 9         | 173 ± 8       |
| Weight, kg      | 74 ± 15         | 74 ± 15         | 80 ± 15       |
| Present or ex-smokers, n (%) | 19 (54%) | 18 (47%) | 7 (64%) |
| Alcohol: Viral Hepatitis: Others | — | 20: 10: 8 | 8: 2: 1 |
| Child-Pugh Grade (A: B: C) | — | 5: 13: 20 | 0: 4: 7 |
| Dyspnea, n (%)  | 0              | 5 (13%)         | 11 (100%)     |

Applicable values are means ± SD. HPS, hepatopulmonary syndrome. *P < 0.0001 vs. non-HPS.
mality may account for the frequent finding of low TLCO in liver cirrhosis even after liver transplantation (12). However, the relationship between low TLCO and arterial oxygenation abnormalities is variable in cirrhotic patients. A strong negative correlation between PA-aO2 and TLCO has been reported in HPS patients (11, 12). By contrast, no correlation was found between TLCO and PA-aO2 in a series of 77 cirrhotic patients in non-HPS patients (22) and in advanced stages of HPS (4, 6, 22). By definition, a diffusion limitation is present when the end-capillary partial pressure of an exchanging gas is not equal to its alveolar value in any capillary vessels, which may contribute to decreased Vcap. Our finding accounted, at least in part, for the decrease in Vcap in our subgroup of HPS patients. The three generally recognized causes of arterial oxygenation abnormalities in cirrhotic patients are alveolar ventilation-perfusion (VA/Q) imbalance, increased intrapulmonary shunt, and diffusion impairment to oxygen, reflecting a diffusion-perfusion defect and/or increased thickness of alveolo-capillary walls (11, 21).

VA/Q imbalance is the pivotal mechanism of arterial oxygenation abnormalities both in non-HPS patients (22) and in patients with HPS (12). The increase in blood flow perfusing lung units with low VA/Q is ascribed to an alteration in the regulation of the pulmonary microcirculation and of the pulmonary vascular tone in liver cirrhosis (4). The alteration of the vascular tone seems partly secondary to a reduced hypoxic pulmonary vasoconstriction, but other yet unknown alterations of humoral mediation are likely to be involved (18, 22). Because pulmonary artery pressure and pulmonary capillary wedge pressure are generally low in cirrhotic patients although cardiac output is high (5, 18, 22), a reduced vascular tone is likely to be present both in precapillary and in postcapillary vessels, which may contribute to decreased Vcap. Our finding of a decreased Vcap and of a negative correlation between Vcap and PA-aO2 is therefore compatible with the hypothesis of a decreased pulmonary vascular tone as a leading cause of VA/Q imbalance in cirrhotic patients.

Although its reality remains speculative (25), the presence of intrapulmonary shunt is suggested by the multiple inert gas elimination technique (MIGET) (21). Intrapulmonary shunt (i.e., nonventilated or zero VA/Q pulmonary units) by deriving blood from normally ventilated pulmonary units may decrease TLCO by decreasing Vcap. Thus intrapulmonary shunt may account, at least in part, for the decrease in Vcap in our subgroup of HPS patients.

Alveolar-capillary diffusion limitation for oxygen has been described as a cause of oxygenation abnormality and low TLCO in cirrhotic patients, especially in patients with advanced stages of HPS (4, 6, 22). By definition, a diffusion limitation is present when the end-capillary partial pressure of an exchanging gas is not equal to its alveolar value in any homogeneous lung region (26). Because inert gases used for MIGET analysis reach equilibration between blood and alveolar gas about ten times faster than oxygen does (and also than carbon monoxide does), inert gases are most often not diffusion limited even when oxygen and carbon monoxide are.

Table 2. Blood gas analysis and lung function measurements in healthy subjects (controls) and in cirrhotic patients with or without HPS

| Patients, n | Controls | PA-aO2 <15 mmHg | PA-aO2 ≥15 mmHg | HPS
|---|---|---|---|---
| n | 35 | 24 | 14 | 11 |
| PaO2, mmHg | 105±7 | 87±6 | 67±13† | 35±4 |
| PaCO2, mmHg | 33±3 | 32±5 | 39±13‡ | 77±10|
| pH | 7.45±0.3 | 7.45±0.04 | 7.46±0.03 | 7.46±0.03 |
| PA-aO2, mmHg | 5±4 | 21±5 | 39±13‡ | 77±10 |
| TLC, %pred | 81±7 | 80±6 | 79±8 | 77±10 |
| FEV1/VC, % | 99±4 | 98±13 | 100±15 | 92±14 |
| RV/TLC, % | 35±4 | 36±4 | 37±3 | 34±5 |
| VA, l | 5.8±1.0 | 5.7±0.7 | 5.5±1.3 | 5.5±1.4 |
| VA, %TLC | 97±3 | 97±3 | 96±5 | 96±5 |
| TLCO, ml·min⁻¹·mmHg⁻¹ | 27.2±3.8 | 24.3±5.9* | 23.4±3.8* | 18.0±6.2** |
| TLCO, %pred | 100±2 | 91±15* | 85±16* | 63±18**‡ |
| TNO, ml·min⁻¹·mmHg⁻¹ | 118.7±20.2 | 101.2±25.4* | 107.0±21.9* | 84.9±25.7**‡ |
| TNO:TLCO Ratio | 4.34±0.33 | 4.26±0.44 | 4.53±0.37†‡ | 4.82±0.72†‡ |
| Vcap, ml | 88±11 | 81±16* | 73±12* | 56±21†‡ |
| Vcap, %pred | 102±23 | 93±15* | 81±14† | 60±21†‡ |
| DmCO, ml·min⁻¹·mmHg⁻¹ | 150±10.3 | 53.9±11.1* | 52.4±11.8* | 43.1±13.0* |
| DmCO, %pred | 100±10 | 89±14* | 88±18* | 83±20* |

Applicable values are means ± SD. PaO2, partial pressure of arterial oxygen; PaCO2, partial pressure of arterial carbon dioxide; pH, arterial pH; PA-aO2, alveolar-arterial oxygen tension difference; FEV1, forced expiratory volume in one second; VC, vital capacity; TLC, total lung capacity; %pred, percentage of the predicted value; RV, residual volume; VA, alveolar volume; TLCO, transfer factor of the lung for carbon monoxide; TNO, transfer factor of the lung for nitric oxide; Vcap, capillary lung volume; DmCO, membrane transfer capacity for CO. *P < 0.05 vs. controls; †P < 0.05 vs. non-HPS patients with PA-aO2 ≤15 mmHg; ‡P < 0.05 vs. non-HPS patients with PA-aO2 ≥15 mmHg.
have been suggested in advanced cirrhosis. Structural pulmonary vascular changes such as collagenous thickening of the alveolo-capillary walls, leading to an approximately twofold increase in the minimum blood-gas distance, have been suggested to contribute to low TLCO (3, 13). Our results are counter to this hypothesis. According to the interpretation of our measurements (7), increased thickening of alveolo-capillary walls would in fact have led to both a decrease in DmCO and a decrease in the TLNO:TLCO ratio, and these were not observed in our patients with low TLCO. Diffusion limitation for oxygen was also thought to occur in cirrhotic patients because the distance between the alveoli and the red blood cells in the central stream of dilated pulmonary microvessels is too great for complete equilibration of oxygen (and also, presumably, equilibration of carbon monoxide) with Hb (20). This would mean that the greater the increase in the thickness of the capillary blood layer, the greater the increase in PA-aO2 and the decrease in TLCO. Our results are in contradiction with this hypothesis because we found a positive correlation between PA-aO2 and the TLNO:TLCO ratio, which means that the greater the increase in PA-aO2, the lower the thickness of the capillary blood layer, the alveolar membrane thickness being normal. Thus an alternative explanation for diffusion limitation may be a reduced transit time of the red blood cells in the lungs related to a high cardiac output frequently seen in cirrhotic patients (5). Peripheral and splanchnic vasodilatation, which cause hyperdynamic circulatory syndrome, may in addition contribute to decreased lung capillary blood volume (16). Our results concord with such a hypothesis.

Limitations of the study. Several conditions associated with liver cirrhosis may have affected our results. Ascites, hepatomegaly, interstitial pulmonary edema, and/or consequences of tobacco smoking may potentially alter TLCO in cirrhotic patients. Significant ascites and/or hepatomegaly have been shown to decrease lung volumes in cirrhotic patients (2). If present, ascites and/or hepatomegaly were only moderate in the patients of our series because all spirometric values were close to predicted values. Similarly, interstitial edema is unlikely to have significantly contributed to low TLCO because DmCO showed a much smaller decrease than TLCO. In addition, significant interstitial edema would have been accompanied by a decrease of the TLNO:TLCO ratio (7). In our series, low DmCO was accompanied by an increase of the TLNO:TLCO ratio; this suggests that a lower DmCO was more likely secondary to decreased recruitment than to an increase in membrane thickness. Airway obstruction secondary to tobacco smoking is another potential confounding factor, as it has been suggested to potentially decrease TLCO (22). Although small airway dysfunction was not specifically investigated in our study, cirrhotic patients had similar forced expiratory volume in 1 s, forced expiratory volume in 1 s/vital capacity ratio, and residual volume/total lung capacity ratio to controls, and we failed to find an association between reduced TLCO and former or present use of tobacco. This is in agreement with previous findings in cirrhotic patients, even in the case of small airway dysfunction (3, 22).

Conclusion. In summary, lower TLCO and increased PA-aO2 were strongly associated with decreased lung capillary blood volume in cirrhotic patients. In addition, a high TLNO:TLCO ratio in patients with oxygenation abnormalities suggests decreased thickness of the capillary blood layer in these patients.
patients. A larger, multicenter study to measure NO and CO lung transfer may help determine the diagnostic value of this test in patients with advanced liver cirrhosis.

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