

Elevated exhaled nitric oxide in patients with hepatopulmonary syndrome

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ABSTRACT: The hypoxaemia of hepatopulmonary syndrome, seen in severe chronic liver dysfunction, occurs as a result of precapillary pulmonary arterial dilatation and arteriovenous communications. These abnormalities contribute to the mismatch between ventilation and perfusion, and the right to left blood flow shunting. Nitric oxide (NO) is a powerful vasodilator concerned with the regulation of pulmonary vascular tone in man.

Using a chemiluminescence analyser, we have measured endogenously produced NO in the exhaled air of three patients with the hepatopulmonary syndrome, six normoxaemic cirrhotic patients and six healthy volunteers. The subjects breathed NO-free air throughout the measurements.

The molar rate of production of exhaled NO was raised almost threefold in the patients with hepatopulmonary syndrome compared with normal volunteers and with normoxaemic cirrhotic patients. Hypoxia *per se*, achieved in the normal volunteers by breathing a hypoxic gas mixture, reduced rather than increased the exhaled NO. One hepatopulmonary syndrome patient received an orthotopic liver transplant and achieved normoxaemia after 3 months. The exhaled NO also returned to normal.

Increased pulmonary production of NO could contribute to the development of the hepatopulmonary syndrome.

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The hepatopulmonary syndrome develops in some patients with severe chronic liver dysfunction of varied aetiology. It is characterized by profound hypoxaemia, which worsens when rising from supine to upright posture, so-called orthodeoxia [1]. Associated with the syndrome there are pulmonary arteriovenous communications and dilatation of precapillary pulmonary arteries [2]. This causes right-to-left shunting of blood flow and mismatch between ventilation and perfusion [3]. Increased endogenous production of nitric oxide (NO) has been proposed as an important determinant of the hyperdynamic circulation of the cirrhotic patient [4]. In many mammalian species, including man, endothelial NO contributes to the normally low pulmonary vascular tone [5]. Inhaled NO is a potent pulmonary vasodilator, which acts selectively on the pulmonary circulation being rapidly inactivated by haemoglobin [6]. It is now possible to measure NO produced in the lung [7, 8] and upper airway [9] from the concentration of NO in the exhaled air. We have used this approach to determine whether the patient with hepatopulmonary syndrome produces an excess amount of NO in the lung.

Subjects and methods

Our local hospital had given ethical approval for the study. Twelve healthy volunteers, six patients with cirrhosis and three patients with cirrhosis and severe hypoxia were studied. All the cirrhotic patients were awaiting orthotopic liver transplantation, selection criteria have been reported in full previously [10]. The liver biopsies showed evidence of chronic active hepatitis (n=2), cryptogenic cirrhosis (n=5), primary biliary cirrhosis (n=1) and primary sclerosing cholangitis (n=1). No patient with evidence of airways obstruction was included. The severe hypoxaemic cirrhotics all showed evidence of orthodeoxia and of intrapulmonary shunting.

The measurement of exhaled NO was undertaken with the subjects semirecumbent. They were asked to inhale and exhale through a two-way non-rebreathing valve; their noses were blocked with nose clips throughout the measurement. For 10 min, they inhaled NO-free air (British Oxygen Company (BOC) Specialist Gases Ltd; Guildford, Surrey, UK), which was held in a 100 L Douglas bag. Sampling of the Douglas bag showed that it contained less than 1 part per billion (ppb) NO. The

exhaled air was collected in another 100 L Douglas bag during the 10 min period. The normal volunteers also breathed a NO-free hypoxic gas mixture of 10% oxygen (O_2) for a 10 min period, during which the exhaled air was collected. During the hypoxic gas breathing, the arterial O_2 saturation (S_{a,O_2}) was monitored with a pulse oximeter (Minolta, DeVilbiss, Middlesex, UK).

The concentration of exhaled NO was measured with a chemiluminescence analyser (model 42, Thermolectron, Warrington, UK). It was calibrated against standard gas mixtures to enable measurements of NO, with a sensitivity of 0.5 ppb. The minute exhaled volume was measured using a Wright's Respirometer (BOC, Guildford Surrey, UK). This device was in circuit with the expiratory port of the two-way valve. The molar rate of production of NO by the lung was calculated by multiplying the concentration of exhaled NO by 10^9 to convert to fractional concentration. This is then multiplied by the minute volume, after correcting for atmospheric pressure and temperature.

All patients had chest radiographs, spirometry and gas transfer for carbon monoxide (TL,CO) undertaken. Right heart cardiac catheterization was performed in the three patients with severe hypoxia. Recordings of mixed venous (C_{v,O_2}) and radial arterial blood (C_{a,O_2}) oxygen contents were made, which were used in the shunt equation: $C_{c,O_2} - C_{a,O_2} / C_{c,O_2} - C_{v,O_2}$, to calculate the shunt fraction, where C_{c,O_2} is the pulmonary capillary oxygen content.

Results were expressed as mean \pm standard deviation (SD). Student's t-test for unpaired data was used to compare differences between groups. The p-values less than 0.05 were taken as significant.

Results

The cirrhotic patients without hypoxaemia had clear chest radiographs and normal lung function, including TL,CO (table 1). Pulse oximetry values ranged 95–97% (table 1). The hypoxaemic patients had reduced TL,CO values (table 1), but normal chest radiographs. They were hypoxaemic and had significant shunt fractions (table 1).

In the semirecumbent position, the normal subjects and the nonhypoxaemic cirrhotics had normal values of exhaled

NO (table 1). By contrast, the hypoxaemic cirrhotics had twice the amount of NO in the exhaled breath (table 1) compared with normals and the nonhypoxaemic cirrhotics. Three months after orthotopic liver transplantation, one hypoxaemic patient had normal arterial blood gas tensions and the exhaled NO production had been restored to normal.

Exposure of the normals to a hypoxic gas mixture caused a fall in the S_{a,O_2} from 98 ± 2 to $81 \pm 4\%$, and a slight fall in the rate of production of NO by the lung from 3.3 ± 1.6 to 2.8 ± 1.6 $nM \cdot min^{-1}$.

Discussion

In this small study, hypoxaemic patients with cirrhosis had elevated rates of production of NO in their lungs.

Our method of measurement allows for the slow response time for the chemiluminescence analysers [11]. Single-breath analysis, whilst having been advocated as a simple measurement [12], is not possible with current technology. Normal subjects and the nonhypoxaemic cirrhotics had exhaled values for NO similar to earlier reports [5].

We have previously shown that mixed exhaled NO level correlates with the TL,CO [13]. There is strong evidence from the isolated perfused and ventilated lungs that the mixed exhaled NO level reflects the rate of pulmonary vascular production [14]. Hypoxaemia *per se* is an unlikely cause of the elevated NO in the hypoxaemic cirrhotics, as in normals hypoxaemia was associated with a slight fall in exhaled NO. This is in keeping with the observed fall in the exhaled NO levels in the isolated lung with hypoxaemia [14] and those observed *in vivo* in guinea-pigs [7]. From these observations, we would have anticipated that the hypoxaemic cirrhotic patients would have had reduced exhaled NO levels. The converse was the case, suggesting increased production of NO in the lungs of patients with hypoxaemia and cirrhosis. This idea is supported by the restoration of a normal level of NO in the one patient who underwent successful orthotopic liver transplantation in association with return to normoxaemia. Liver transplantation can reverse the ventilation-perfusion mismatch seen in the hepatopulmonary syndrome and restore normoxaemia [15].

Table 1. – Lung function, arterial blood gases and rate of production of nitric oxide

	Hepatopulmonary patients			Cirrhotic controls	Normal controls	
	No. 1 Breathing air	No. 2 Breathing air	No. 3 Breathing air	(n=6) Breathing air	(n=18) Breathing air	(n=12) Breathing 10% O_2
TL,CO $mmol \cdot kPa^{-1} \cdot min^{-1}$	5.4	4.93	3.35	6.71 ± 0.3	8.6 ± 0.5	-
P_{a,O_2} kPa	8.5	6.6	8.2	13.2 ± 1.6	-	-
S_{a,O_2} %	93	85	91	95–97	96–100	70–87
Shunt fraction %	21	26	17	-	-	-
MNO $nM \cdot min^{-1}$	9.2	8.7	12.5	3.4 ± 1.8	3.3 ± 1.8	2.8 ± 1.6
MNO after transplantation $nM \cdot min^{-1}$	4.7	-	-	-	-	-

TL,CO : transfer factor of the lungs for carbon monoxide; P_{a,O_2} : arterial oxygen tension; S_{a,O_2} : arterial oxygen saturation; MNO: Molar rate of production of nitric oxide.

Nitric oxide is produced by a network of cells in the lungs [16]. It is not possible in our study to define the site of increased production in the hypoxaemic cirrhotics. The calcium independent nitric oxide synthase (iNOS) can be induced to produce nanomolar amounts of NO in most cell types [17]. This includes epithelial cells in asthmatics [18]. NO is increased in exhaled air of asthmatics [19]. Induction of iNOS in endothelial cells by cytokines and endotoxin has been suggested as the mechanism of the hyperdynamic circulation of cirrhosis [4]. Levels of circulating endotoxin [20] and cytokines [21, 22] are increased in cirrhotics. Indeed, enhanced NO production both in the systemic and pulmonary circulation could account for the reduced vascular tone seen in both circulations of cirrhotics [23]. However, we observed elevated values of exhaled NO only in the patients with hypoxaemia. The small size of our study could account for these findings, or alternatively it can be argued that the hypoxaemia in cirrhosis and elevated exhaled NO are specifically linked. This interesting finding requires further investigation, but possibly offers a clue to the mechanism causing the hepatopulmonary syndrome.

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