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Correlation of inhaled nitric-oxide induced reduction of pulmonary artery pressure and vascular changes

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Correlation of inhaled nitric-oxide induced reduction of pulmonary artery pressure and vascular changes. B.H. Jiang, J. Maruyama, A. Yokochi, H. Amano, Y. Mitani, K. Maruyama. ©ERS Journals Ltd 2002.

ABSTRACT: The purpose of the present study was to determine the relationship between hypertensive pulmonary vascular remodelling and the changes in mean pulmonary artery pressure (mPAP) during low-dose nitric oxide (NO) inhalation.

Rats were exposed to chronic hypobaric hypoxia (air at 50.5 kPa (380 mmHg), 10% oxygen, for 5–29 days) to induce chronic pulmonary hypertension (PH) with pulmonary vascular structural changes. After the chronic hypoxic exposure, the rats had an indwelling pulmonary artery catheter inserted and changes in mPAP with NO were correlated to morphometrical analysis of pulmonary vascular changes.

All concentrations of inhaled NO (0.1–2.0 parts per million) reduced mPAP with a similar per cent reduction from baseline mPAP in PH rats, while no changes were observed in control rats. During NO inhalation in PH rats, the absolute value of the decrease in mPAP, but not per cent reduction in mPAP, significantly correlated with baseline mPAP, the percentage of muscularised arteries at the alveolar wall level and at the alveolar duct level, and the per cent medial wall thickness of muscularised arteries.

In the chronic hypoxic pulmonary hypertension model, the severity of pulmonary vascular remodelling did not alter the reactivity of the pulmonary arteries to nitric oxide and might, in part, determine the magnitude of nitric-oxide induced absolute reduction in mean pulmonary artery pressure.

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In all conditions causing pulmonary hypertension (PH), vascular changes include new muscularisation of normally non-muscular peripheral pulmonary arteries and medial muscle hypertrophy of normally muscular arteries. Abnormal muscular pulmonary vasculature might have a heightened reactivity [1, 2], which may explain the effect of nitric oxide (NO) inhalation in hypertensive pulmonary disease, because NO dilates constricted pulmonary vasculature. Although inhaled NO can dilate these structurally-remodelled pulmonary arteries, the degree of response to inhaled NO was quite variable in patients with congenital heart disease [1], primary pulmonary hypertension (PPH) [3], limited scleroderma and isolated PH [4] and severe acute respiratory distress syndrome (ARDS) [5]. The differences in severity of pulmonary vascular structural changes might explain the differences in inhaled NO-induced reduction in pulmonary artery pressure (PAP) among patients.

Chronic hypoxia induces PH and hypertensive pulmonary vascular changes in rats [6–8]. With increasing exposure to hypoxia, there is a progressive increase in the severity of the hypertensive pulmonary vascular changes [7]. These findings allowed the authors to make a rat model of experimentally different degrees of PH and hypertensive pulmonary vascular changes. To determine the relationship between the hypertensive pulmonary vascular changes and response to inhaled NO, NO inhalation was carried out in a rat model of chronic PH induced by chronic hypoxic exposure of varying durations. Quantitative morphometrical analysis was applied to determine the severity of hypertensive pulmonary vasculature [8–10]. Low-dose NO inhalation $\leqslant$2 parts per million (ppm) was chosen because a lower dose is favourable to minimise any possible toxicity.

Materials and methods

Forty male Sprague-Dawley rats weighing 250–400 g (Clea, Shiga, Japan) were used. Rats were exposed to hypobaric hypoxia (air at 50.5 kPa (380 mmHg), 10% oxygen) for 5 days (n=12), 10 days (n=6), 11–20 days (n=9) and 21–29 days (n=5). Eight control rats were kept in room air without hypoxic exposure. After the hypoxic exposure period, the rats were anaesthetised with pentobarbital sodium (45 $mg \cdot kg^{-1}$) given intraperitoneally. A pulmonary artery catheter (Silastic tubing, 0.31 mm inside diameter (ID) and 0.64 mm outside diameter (OD)) was inserted *via* the right external jugular vein into the pulmonary artery by a closed-chest technique, as described previously [8, 10]. The catheter was then

tunnelled subcutaneously and exteriorised in the middle region of the scapular of the rat. After catheterisation, the rats were kept in room air only. After 24–48 h following catheterisation, with the rats fully awake, each rat breathed a series of NO and air mixtures, containing 0.1, 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0 ppm NO for 15 min in the exposure chamber (the ambient concentration of NO was 0.01 ppm in the laboratory air), during which time an extension tube (SP-19; Natsume, Tokyo, Japan) was connected to the pulmonary artery catheter and passed to the outside of the chamber through a rubber cap. Pressure was recorded with a physiological transducer and an amplifier system (AP 620G; Nihon Kohden, Tokyo, Japan) before, during, and after NO inhalation, in which mean pulmonary artery pressure (mPAP) was obtained electronically. The pressure was recorded while the rats were quiet. Each level of NO exposure was followed by 15 min of breathing air only. The absolute value of the reduction in mPAP was obtained:

$$\text{mPAP without NO} - \text{mPAP on NO inhalation} \quad (1)$$

The per cent reduction from baseline mPAP was also calculated:

$$\frac{(\text{mPAP without NO} - \text{mPAP on NO})}{\text{baseline mPAP} \times 100\%} \quad (2)$$

An aortic catheter was also passed from the left carotid artery into the ascending aorta in another set of rats and mean systemic arterial pressure (mSAP) was obtained.

Nitric oxide exposure chamber

NO was obtained from Sumitomo Seika (Chiba, Japan) as a mixture of 89.1 ppm in pure N_2. This mixture was introduced into the tube connected to a plastic exposure chamber (~2 L capacity), in which 3 $L \cdot min^{-1}$ constant airflow was developed. The constant airflow was obtained with an air compressor (0.4 OP-7S; Hitachi, Tokyo, Japan) and flow meter. Thus, before NO was introduced into the exposure chamber, the gas was diluted in the upstream of the chamber with fresh air and the oxidation of NO to NO_2 in the exposure chamber was avoided as far as possible. The NO concentration in the chamber was measured using a chemiluminescence analyser (CLA-510SS; Horiba, Kyoto, Japan). The limitations of resolution of the chemiluminescence analyser depended on the selected range of measurement: 0.01 ppm for a range of 0–l ppm and 0.1 ppm for 0–10 ppm. The pressure gauge attached to the NO and N_2 cylinder was adjusted to obtain the final desired NO concentration in the exposure chamber.

Preparation of lung tissue for structural analysis

After the haemodynamic measurement, the rats were mechanically ventilated through a tracheotomy under pentobarbital sodium anaesthesia. A midline sternotomy was performed to expose the heart and lungs. A blood sample for the haematocrit was obtained from the heart. The main pulmonary artery was cannulated with polyethylene tubing (SP-110; Natsume, Tokyo, Japan) and perfused with saline at a pressure of 20 cmH_2O to clear the blood. The pulmonary artery was then injected with a hot (60°C) radiopaque barium-gelatin mixture at a pressure of 100 cmH_2O, until snowflakes were obtained on the surface of the lung. The pulmonary artery cannula was clamped and the heart and lungs were removed *en bloc*. The lung was distended and fixed by perfusion through the tracheal tube with 10% formalin at a pressure of 36 cmH_20 for 72 h. A block of tissue obtained from the midsection of the left lung parallel to the hilum was processed for light microscopy by paraffin embedding. Sections were stained for elastin by the Van Gieson method. The right ventricle (RV) of the heart was dissected from the left ventricle plus septum (LV+S) and weighed separately. The ratio of RV/(LV+S) was calculated.

Light microscopy slides were analysed using techniques described previously [8–10]. All barium-filled arteries in each tissue section were examined at a magnification of ×400, for an average of 320 $arteries \cdot section^{-1}$ (172–502 $arteries \cdot section^{-1}$). Each artery was first identified, according to its accompanying airway, as being related to a terminal bronchiolus, respiratory bronchiolus, alveolar duct or alveolar wall. Each artery was also identified as being one of two structural types: muscularised (muscular, with a complete medial coat of muscle or partially muscular, with an incomplete coat, only a crescent of muscle being present), and nonmuscular (no muscle apparent). The percentages of muscularised arteries at the alveolar wall (%AW) and duct (%AD) level were calculated. For all the muscular arteries between 100 μm and 200 μm in diameter (4–33 arteries were found per section), usually accompanied with a terminal or respiratory bronchiolus, the wall thickness of the media (distance between external and internal elastic laminae) was measured along the shortest curvature, and the per cent medial wall thickness (%MWT) was calculated [8].

Morphometrical analyses were performed without previous knowledge of hypoxic exposure durations, baseline PAP and responses to NO inhalation. Only after completion of independent morphometric analysis of all specimens were correlations made with the hypoxic exposure durations and the PAP measurements.

Statistical analysis

All values are presented as mean±SEM. Relationships between hypoxic exposure period and mPAP, RV/LV+S, %AW, %AD, and %MWT were analysed using linear regression analysis. Analysis of variance (ANOVA) for repeated measures was used to analyse mPAP with and without NO inhalation. Concentration-dependent effects of NO on per cent reduction in mPAP were analysed by one-way ANOVA followed by

Scheffe test. Relationships between NO-induced changes in mPAP and morphological parameters (%AW, %AD and %MWT) were analysed using linear regression analysis. A p-value of <0.05 was considered statistically significant.

Results

Induction of pulmonary hypertension

In rats kept in room air, control values without chronic hypoxic exposure were obtained: mPAP was 19.3±0.6 mmHg (n=8); RV/(LV+S) was 0.26±0.03 (n=8); %AW and %AD level were 11.3±1.5% (n=8) and 19.3±2.0% (n=8), respectively; %MWT was 2.03± 0.23% (n=8); and haematocrit was 41.5±3.04% (n=6).

Exposure to chronic hypoxia was associated with a significant increase in the level of mPAP, RV/(LV+S), %AW, %AD, %MWT and haematocrit. With increasing exposure period to hypoxia, mPAP, RV/(LV+S), %AW, %AD, %MWT and haematocrit increased in severity. There was significant positive correlation between mPAP and RV/(LV+S) ($r=0.66$, $p<0.0001$), %AW ($r=0.81$, $p<0.0001$), %AD ($r=0.81$, $p<0.0001$), and %MWT ($r=0.73$, $p<0.0001$).

Nitric oxide inhalation

Short-term NO inhalation significantly reduced mPAP in PH rats exposed to hypoxia for 5 days (n=12), 10 days (n=6), 11–20 days (n=9) and 21–29 days (n=5) at all concentrations from the smallest concentration of 0.1 ppm, but not in control rats (repeated measures of ANOVA) (fig. 1). The per cent reductions in mPAP were not significantly different among these four experimental groups at each concentration of inhaled NO, so values from all rats at the same inhaled concentration were combined to determine the dose-dependent effect of inhaled NO (0.1, 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0 ppm) on per cent reduction from baseline mPAP. There was no significant dose dependency of inhaled NO concentration on per cent reduction in mPAP in the range between 0.1–2.0 ppm (ANOVA), so that the absolute value of the reduction in mPAP during NO inhalation in each rat was expressed as the average value of the changes at all concentrations of NO. The absolute value of the decrease in mPAP during NO inhalation significantly correlated with the baseline mPAP ($r=0.58$, $p=0.0005$) (fig. 2).

Comparisons of morphometry and inhaled nitric-oxide induced changes in mean pulmonary artery pressure

No correlation was observed between the per cent reduction in mPAP and %AW, %AD, and %MWT. There was significant positive correlation between the absolute value of NO-induced reduction in mPAP and the percentage of muscularised peripheral pulmonary arteries (%AW, $r=0.58$, $p=0.0009$; %AD, $r=0.45$, $p=0.015$) (fig. 2). There was also a slight but significant weak positive correlation between the NO-induced reduction in mPAP and medial hypertrophy of the muscular artery ($r=0.38$, $p=0.04$) (fig. 2).

Aortic pressure

There was no change in aortic pressure with 5 ppm NO inhalation in PH rats (n=4) (12.40±0.98 kPa (93.3±7.4 mmHg) without NO; 12.40±0.88 kPa (93.3± 6.6 mmHg) with NO).

Discussion

The absolute value of the decrease in mPAP with NO inhalation, but not per cent reduction in mPAP, was significantly correlated with baseline mPAP and morphological values of vascular changes: %AW, %AD and %MWT. The higher the baseline mPAP, the greater the absolute value of the reduction in mPAP with NO inhalation. The greater the abnormal extension of smooth muscle to peripheral arteries, the greater the reduction of mPAP in absolute value with NO inhalation.

Pressure is dependent on blood flow and vascular resistance. Although PAP depends upon cardiac output, measurement of cardiac output in conscious rat during NO inhalation is difficult. The authors measured mSAP through the indwelling aortic catheter during and after the NO inhalation at 5 ppm in some rats. Because no significant changes in mSAP during NO inhalation were found and because inhaled NO is a selective pulmonary vasodilator [1, 3–5], it was assumed that the cardiac output remained unaffected with NO inhalation, even though it was not measured in the present experiment. The previous study in patients with PH also reported no changes in the values of cardiac output and SAP during NO inhalation [11].

Inhaled NO significantly reduced mPAP in PH rats, but not in normal rats. In normal rats, pulmonary vasculature might be maximally dilated. The absolute value of reduction in mPAP and per cent reduction from baseline mPAP was less than that reported in earlier clinical reports [12, 13], suggesting that the reversible component plays a minor role in the increase in PAP in chronic hypoxic PH. Fixed vascular structural changes might contribute to the increase in mPAP. Since NO inhalation was performed in room air more than 24 h after weaning from chronic hypoxic exposure, there should be little hypoxic pulmonary vasoconstriction. Although the intrinsic tone (reversible component) was small, this component was significantly correlated with the vascular changes. Inhaled NO reduced vascular tone that might be intrinsic in hypertensive pulmonary vasculature.

Generally, a polycythaemia follows chronic hypoxic exposure and plays an important role in hypoxic PH in rats and humans. The authors measured the haematocrit value and found that there was a progressive increase in haematocrit with increasing exposure to hypoxia (0 days: 41.5%; 10 days: 58.0%;

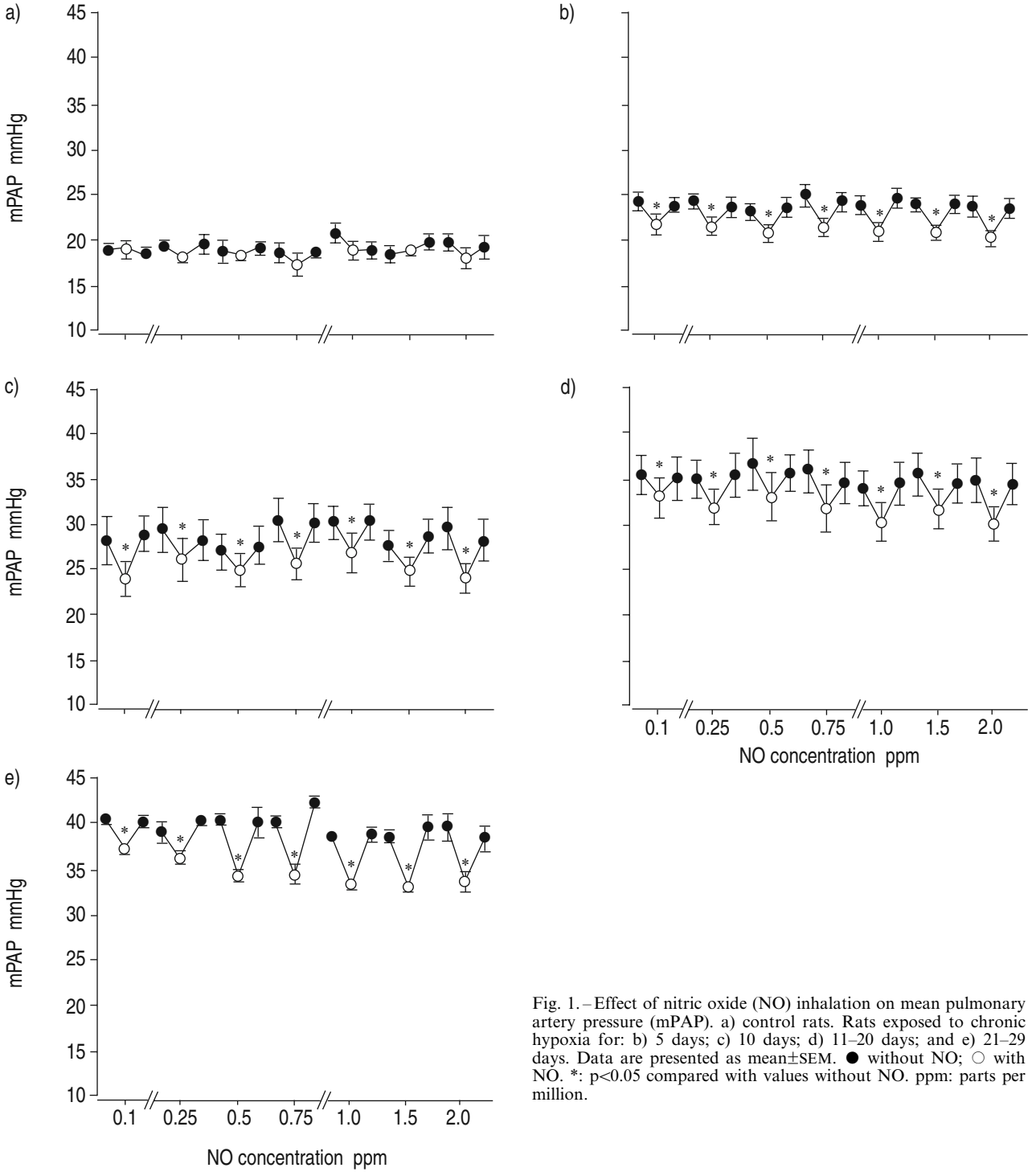

Fig. 1. – Effect of nitric oxide (NO) inhalation on mean pulmonary artery pressure (mPAP). a) control rats. Rats exposed to chronic hypoxia for: b) 5 days; c) 10 days; d) 11–20 days; and e) 21–29 days. Data are presented as mean±SEM. ● without NO; ○ with NO. *: $p<0.05$ compared with values without NO. ppm: parts per million.

11–20 days: 60.1%; and 21–29 days: 68.6%). It was also found that there were similar percentage reductions in mPAP during NO inhalation from 0.1–2.0 ppm. It was therefore speculated that polycythaemia did not decrease the relaxant effect of NO in rats in the present study.

The MWT shows the hypertrophy and hyperplasia of smooth muscle cells. One study has suggested that pulmonary artery smooth muscle hypertrophy and hyperplasia are associated with pulmonary vasoconstriction [1]. New muscularisation of normally non-muscular peripheral arteries shows that pericytes and intermediate cells differentiate into mature smooth muscle cells. The contractile ability to extrinsic vasoconstrictors in these newly muscularised arteries has not been studied extensively. DAVIES *et al.* [14] reported that the response to vasoconstrictor in newly muscularised arteries is reduced compared

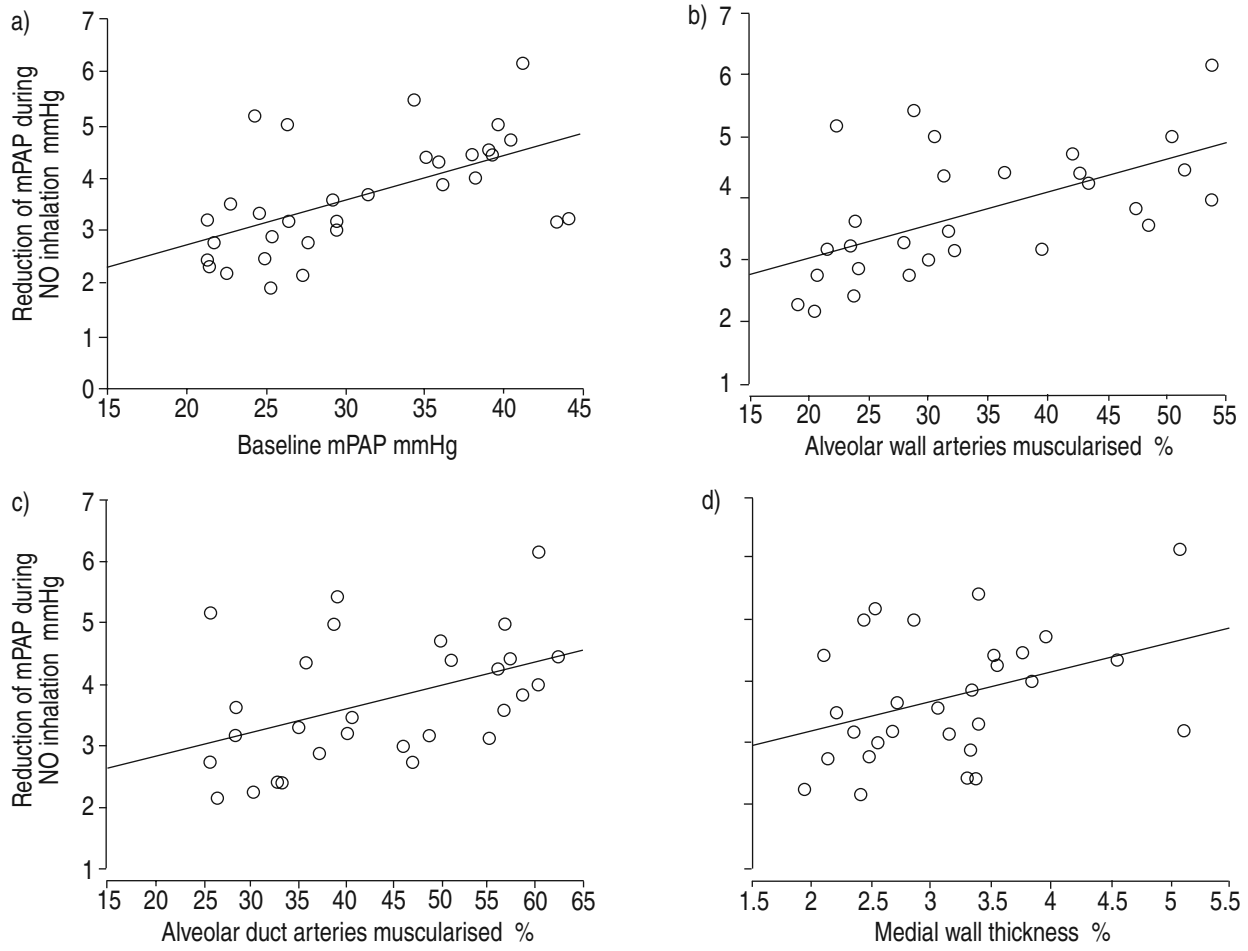

Fig. 2. – Absolute value of the reduction in mean pulmonary artery pressure (mPAP) during nitric oxide (NO) inhalation, the baseline mPAP and the severity of the vascular remodelling. a) Baseline mPAP (r=0.58, p=0.0005), b) alveolar wall arteries muscularised (r=0.58, p=0.0009), c) alveolar duct arteries muscularised (r=0.45, p=0.015) and d) medial wall thickness (r=0.38, p=0.04).

with normal peripheral arteries of the same size. Since NO-induced reduction in mPAP is positively correlated with the level of abnormal muscularisation of peripheral pulmonary arteries, newly muscularised arteries would have intrinsic vascular tone and thus, the total vasoconstrictive area would increase with the level of extension of smooth muscle into the peripheral pulmonary artery. Abnormal PAP correlates best with abnormal extension of muscle into peripheral arteries [8], which is consistent with the present findings. A new finding is that the degree of abnormal muscularisation of peripheral arteries is also significantly correlated with inhaled NO-induced absolute reduction in mPAP.

An increase in reactivity to NO in this model would be best assessed by the per cent decrease in pulmonary vascular resistance (PVR). However, as PVR was not measured and because it was assumed that the cardiac output remained unaffected, the reactivity was assessed by the per cent decrease in PAP rather than by the absolute change in PAP with NO. It was concluded that abnormal extension of smooth muscle to peripheral arteries did not increase the reactivity of the pulmonary arteries to NO. Many studies show a decrease in endothelium-dependent relaxation and in endothelium-independent relaxation in the pulmonary arteries of chronically hypoxic rats [15]. Conversely, Dinh-Xuan *et al.* [16] and Orton *et al.* [17] demonstrated that endothelium-independent vasodilator response to sodium nitroprusside was unaltered.

Since abnormally muscular pulmonary vasculature might have a heightened reactivity [18], this suggested that the per cent reduction in mPAP due to NO inhalation would increase with the increase in severity in vascular changes. However, this was not the case in the present study. Under unstimulated conditions, hypertensive pulmonary vasculature might have a similar baseline vascular tone regardless of the severity of vascular changes, leading to similar per cent reductions in mPAP due to NO inhalation. Because of the similar per cent reductions at each concentration of NO, the higher the baseline mPAP, the greater the absolute value of the reduction in mPAP was. Although absolute active force-generating ability is increased due to the increase in the amount of smooth muscle cells, contractile force per cross-sectional area is reduced in hypertensive pulmonary arteries [19].

It was estimated that less NO-induced response

suggests less severe vascular changes in the chronic hypoxic PH. However, this might not be the case in the clinical situation of hypertensive pulmonary vascular disease with intimal changes that show a high grade of severity in hypertensive pulmonary vascular disease. The present model does not develop intimal cellular and fibrotic proliferation. Once intimal changes develop, vascular responses to vasodilator start to decrease. The area of media was inversely related to the area of the intima, suggesting that medial wall thickness regresses despite progression of PH. Therefore, one weakness of this study was that the results applied to a very selective model, which were difficult to extend to clinical observations. Failure of inhaled NO to reduce PAP in a given patient with PH may not mean that pulmonary vascular remodelling is low in that particular case. Thus, high baseline PAP and a small response to inhaled NO might suggest fixed vascular changes, such as cellular and fibrous intimal proliferation. High baseline PAP and a good response to inhaled NO might suggest vascular lesions without intimal changes. In biopsy lung specimens from patients with PPH, the greater the area of pulmonary artery media, the greater the changes in PAP induced by intravenous or oral vasodilator drugs [20].

The reduction of mPAP during NO inhalation was correlated with the level of mPAP without NO in ARDS [12, 13, 21], chronic obstructive pulmonary disease [22] and congenital heart disease [18], but not in PPH [3] and another group of ARDS [5]. The degree of response to inhaled NO was quite variable [1]. Although pulmonary vascular response to inhaled NO is probably more complicated than a simple dependency upon the degree of muscularisation, one possible explanation might be the different degree of vascular remodelling among patients.

Nitric oxide inhalation is used for reducing pulmonary hypertension and improving oxygenation. In addition to treatment, nitric oxide inhalation is used in preoperative assessment at cardiac catheterisation to identify patients with a critically-limited and restricted pulmonary vascular bed [18]. Since acute responses to nitric oxide in primary pulmonary hypertension are similar to those of vasodilators, such as prostacyclin, the response to nitric oxide inhalation might also be used to predict the effect of oral administration of these drugs [3]. The present study showed that in a selective model of chronic hypoxic pulmonary hypertension in rats, the severity of pulmonary vascular remodelling might partly determine the magnitude of nitric-oxide induced reduction in mean pulmonary artery pressure.

References

1. Curran RD, Mavroudis C, Backer CL, Sautel M, Zales VR, Wessel DL. Inhaled nitric oxide for children with congenital heart disease and pulmonary hypertension. *Ann Thorac Surg* 1995; 60: 1765–1771.
2. Rabinovitch M, Keane JF, Norwood WI, Castaneda AR, Reid L. Vascular structure in lung tissue obtained at biopsy correlated with pulmonary hemodynamic findings after repair of congenital heart defects. *Circulation* 1984; 69: 655–667.
3. Sitbon O, Brenot F, Denjean A, *et al.* Inhaled nitric oxide as a screening vasodilator agent in primary pulmonary hypertension. *Am J Respir Crit Care Med* 1995; 151: 384–389.
4. Williamson DJ, Hayward C, Rogers P, *et al.* Acute hemodynamic responses to inhaled nitric oxide in patients with limited scleroderma and isolated pulmonary hypertension. *Circulation* 1996; 94: 477–482.
5. Rossaint R, Gerlach H, Schmidt-Ruhnke H, *et al.* Efficacy of inhaled nitric oxide in patients with severe ARDS. *Chest* 1995; 107: 1107–1115.
6. Meyrick B, Reid L. The effect of continued hypoxia on rat pulmonary arterial circulation. *Lab Invest* 1978; 38: 188–200.
7. Hislop A, Reid L. New findings in pulmonary arteries of rats with hypoxia-induced pulmonary hypertension. *Br J Exp Path* 1976; 57: 542–554.
8. Rabinovitch M, Gamble WJ, Miettinen OS, Reid L. Age and sex influence on pulmonary hypertension of chronic hypoxia and on recovery. *Am J Physiol* 1981; 240: H62–H72.
9. Maruyama J, Maruyama K, Mitani Y, Kitabatake M, Yamauchi T, Miyasaka K. Continuous low-dose NO inhalation does not prevent monocrotaline-induced pulmonary hypertension in rats. *Am J Physiol* 1997; 272: H517–H524.
10. Maruyama K, Ye CL, Woo M, *et al.* Chronic hypoxic pulmonary hypertension in rats and increased elastolytic activity. *Am J Physiol* 1991; 261: H1716–H1726.
11. Yoshida M, Taguchi O, Gabazza EC, *et al.* The effect of low-dose inhalation of nitric oxide in patients with pulmonary fibrosis. *Eur Respir J* 1997; 10: 2051–2054.
12. Manktelow C, Bigatello LM, Hess D, Hurford WE. Physiologic determinants of the response to inhaled nitric oxide in patients with acute respiratory distress syndrome. *Anesthesiology* 1997; 87: 297–307.
13. Bigatello LM, Hurford WE, Kacmarek RM, Roberts JD, Zapol WM. Prolonged inhalation of low concentrations of nitric oxide in patients with severe adult respiratory distress syndrome. *Anesthesiology* 1994; 80: 761–770.
14. Davies P, Maddalo F, Reid L. Effects of chronic hypoxia on structure and reactivity of rat lung microvessels. *J Appl Physiol* 1985; 58: 795–801.
15. Hampl V, Herget J. Role of nitric oxide in the pathogenesis of chronic pulmonary hypertension. *Physiol Rev* 2000; 80: 1337–1372.
16. Dinh-Xuan AT, Higenbottam TW, Clelland CA, *et al.* Impairment of endothelium-dependent pulmonary-artery relaxation in chronic obstructive lung disease. *N Engl J Med* 1991; 324: 1539–1547.
17. Orton EC, Reeves JT, Stenmark KR. Pulmonary vasodilation with structurally altered pulmonary vessels and pulmonary hypertension. *J Appl Physiol* 1988; 65: 2459–2467.
18. Roberts JD, Lang P, Bigatello LM, Vlahakes GJ, Zapol WM. Inhaled nitric oxide in congenital heart disease. *Circulation* 1993; 87: 447–453.
19. Griffith SL, Rhoades RA, Packer CS. Pulmonary arterial smooth muscle contractility in hypoxia-induced pulmonary hypertension. *J Appl Physiol* 1994; 77: 406–414.
20. Palevsky HI, Schloo BL, Pietra GG, *et al.* Primary pulmonary hypertension. *Circulation* 1989; 80: 1207–1221.

21. Lowson SM, Rich GF, McArdle PA, Jaidev J, Morris GN. The response to varying concentrations of inhaled nitric oxide in patients with acute respiratory distress syndrome. *Anesth Analg* 1996; 82: 574–581.

22. Moinard J, Manier G, Pillet O, Castaing Y. Effect of inhaled nitric oxide on hemodynamics and VA/Q inequalities in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1994; 149: 1482–1487.