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Surgically induced unilateral pulmonary hypertension: time-related analysis of a new experimental model☆

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Abstract

Objective: Patients with irreversible pulmonary vascular obstructive disease caused by pulmonary hypertension due to congenital heart defects are considered either inoperable or only candidates to lung transplantation. This study evaluated an experimental model of surgically induced unilateral pulmonary hypertension. **Methods**: In eight pigs, 2-months-old, the left pulmonary artery was divided at the origin and end-to-side anastomosed to the descending thoracic aorta through a left thoracotomy. In this way, increased pulmonary blood flow in the right lung and systemic perfusion pressure and oxygenation in the left lung were obtained. After an interval of 6–12 weeks the animals underwent cardiac catheterization and were then sacrificed. Histological examination was done on both the lungs. **Results**: The mean left-to-right shunt through the left pulmonary artery diminished from 58.9 ± 9.6% at the end of the procedure to 4.5 ± 1.5% at the latest hemodynamic evaluation ($P < 0.01$). Pressures and saturations remained identical in aorta and left pulmonary artery, without reduction (NS) with $FiO_2 = 1.0$ ventilation; in the right pulmonary artery there was a mild elevation of the pressures, but still responsive ($P < 0.05$) to $FiO_2 = 1.0$ ventilation. Lung histology showed normal right pulmonary arteries, but irreversible vascular lesions like intimal fibrosis, medial hypertrophy, vascular occlusions, plexiform and dilatation lesions in all the left lungs. **Conclusions**: The lung exposed to systemic pressure and oxygenation develops irreversible vascular lesions typical of pulmonary vascular obstructive disease. The lung exposed to increased flow shows only mild elevation of the arterial pressure, remains responsive to oxygen vasodilatation, and displays normal histology.

Keywords: Congenital heart defects; Eisenmenger syndrome; Irreversible pulmonary vascular lesions; Lung histology; Pulmonary hypertension; Pulmonary vascular obstructive disease

1. Introduction

Survival for patients with Eisenmenger syndrome (irreversible pulmonary vascular obstructive disease with elevated pulmonary vascular resistance and right-to-left shunt as a consequence of a congenital heart defect with a connection between the systemic and the pulmonary circulations) has not improved substantially in the past several decades. The quality of life is universally altered by the presence of cyanosis, exercise intolerance, and the comorbid conditions associated with erythtocytosis such as thromboembolic events and cerebrovascular complications [1–8]. Pregnancy and non-cardiac surgery are associated with high mortality rate in patients with Eisenmenger syndrome [4,5,9,10].

Several experimental models of pulmonary hypertension have been reported in the literature review [11–22], but all of them failed to reproduce a situation with adequate and simultaneous increase of pulmonary blood flow, pressure and oxygen saturation in one lung. In order to reproduce a situation in the pulmonary circulation similar to the Eisenmenger syndrome, we designed an experimental study with the surgical creation of a unilateral pulmonary hypertension, obtained by division of the left pulmonary artery at the origin and its anastomosis end-to-side to the descending thoracic aorta. In this way, we created a model with the left lung perfused with elevated blood flow, systemic pressure and oxygen saturation, while the right lung remained as the control lung, perfused with the same

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pressure and oxygen saturation, but with increased blood flow.

2. Materials and methods

All animals received human care in compliance with the 'Principles of Laboratory Animals' formulated by the National Society of Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication 85-23, revised 1985). The protocol was approved by the institutional Committee on Animal Research.

2.1. Surgical technique

Eight domestic pigs, 2 months of age and with a body weight of 30 ± 2 kg, received i.m. ketamine 15 mg/kg, azaperon 0.5 mg, atropine 2 mg and penicillin G 1,000,000 IU. General anesthesia was induced and maintained with inhaled isoflurane 1.5%, with tracheal intubation and mechanical ventilation. Electrocardiogram (EKG), percutaneous arterial O_2 saturation and expired CO_2 were continuously monitored. Through a left thoracotomy in the fourth intercostal space, the hemiazygos vein was divided, the descending thoracic aorta and the left pulmonary artery were dissected free, and 1 mg/kg of heparin was given i.v. After division of the left pulmonary artery at its origin and suture of the proximal stump, an end-to-side anastomosis was performed with 6/0 polypropylene running sutures between the descending thoracic aorta and distal stump of the left pulmonary artery (Fig. 1A). Vascular clamps were utilized for the temporary control of the left pulmonary artery and the descending thoracic aorta during the confection of the anastomosis. Immediately after opening of the vascular clamps, furosemide 1 mg/kg i.v. was given, and dopamine 5 μg/kg i.v. infusion was started, until the end of the procedure, to prevent or reduce the negative consequences of the suddenly induced unilateral pulmonary edema, observed in the first animal of this experimental study, which died shortly after the tracheal extubation. The accomplishment of an unrestricted anastomosis was verified by the presence of a continuous thrill on the distal left pulmonary artery and by direct pressure measurement in both the aorta and the left pulmonary artery in order to demonstrate identical values. Flow-meters were applied to the descending thoracic aorta and the left pulmonary artery. After insertion of a 24Fr chest drainage, the thoracotomy was closed and the animal was awakened and extubated. The chest drainage remained in place until the whole air in the left pleural space was evacuated (usually 2–4 h). Animals were then sent back to the farm, where they received furosemide 40 mg/day/7 days per os. No anticoagulant or antiplatelet agents were given.

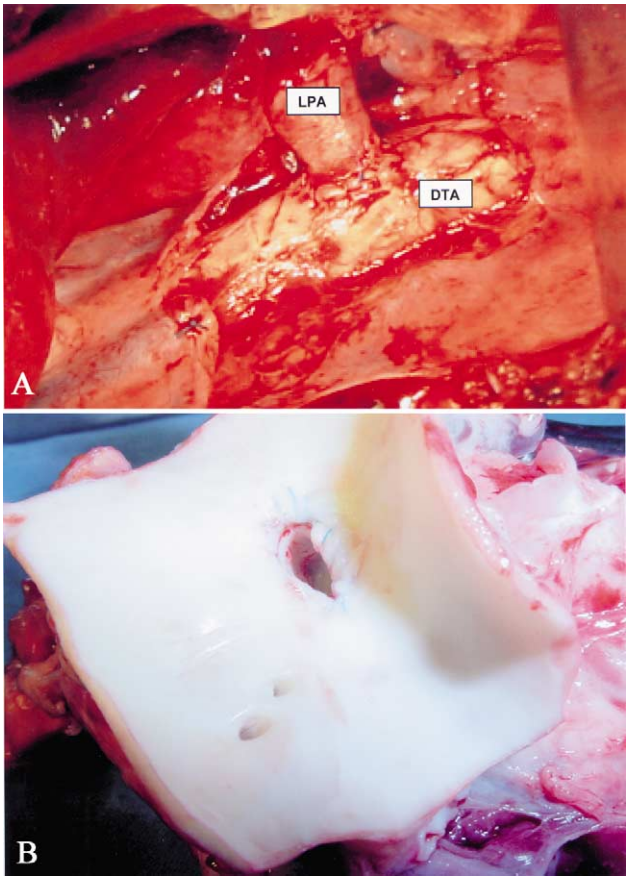

Fig. 1. (A) Intra-operative photograph of the end-to-side anastomosis of the left pulmonary artery (LPA), divided at the origin, and the descending thoracic aorta (DTA). (B) Post-mortem macroscopic aspect of the anastomosis between descending thoracic aorta and left pulmonary artery.

2.2. Assessments

After an interval of 6–12 weeks, under the same general anesthesia and monitoring used for the first procedure, the left carotid artery was isolated and through an 8Fr

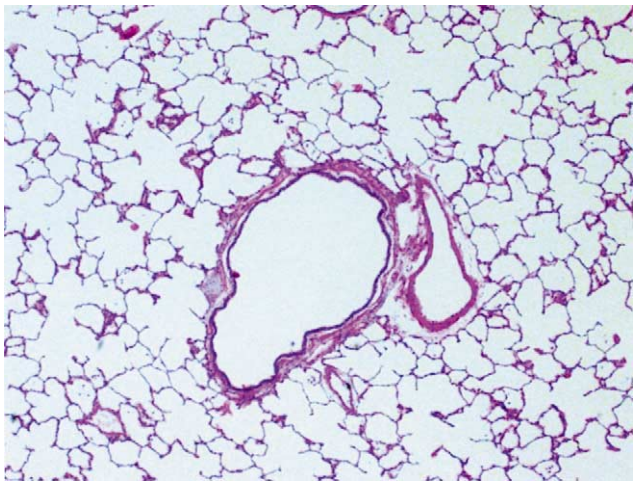

Fig. 2. Histology (van Gieson–elastin stain) of the right lung after 12 weeks, showing a pulmonary artery with normal morphology.

introducer a 6Fr angiography's catheter was inserted to retrograde reach the descending thoracic aorta and the left pulmonary artery. The Intra Vascular Ultra Sound probe was inserted to acquire data on anastomotic cross-sectional area, with the EKG used as a trigger in order to distinguish the systolic versus the diastolic measurements. Color Doppler echocardiography was also performed. After median sternotomy and longitudinal pericardiotomy, flow-meters were applied to the ascending aorta and the main pulmonary artery. Pressures and oxygen saturation were measured on room air ($FiO_2 = 0.21$) and after 30 min with 100% oxygen ventilation ($FiO_2 = 1.0$) in right and left atrium, aorta, left and right pulmonary artery. Vascular resistance through the left lung was calculated with the following formula expressed as Units = mmHg/l/min: (mean left pulmonary artery pressure − mean left atrial pressure) divided by flow through the left pulmonary artery.

After hemodynamic measurements were obtained, the animals were sacrificed. Macroscopic (Fig. 1B)and histologic examinations were done on both lungs and the anastomosis.

Student's *t*-test has been used for statistical evaluation. Data are expressed as mean ± SD. The significance level was $P = 0.05$.

2.3. Instrumentation

2.3.1. Pressure measurement

Pressures were obtained using high-fidelity pressure probe (Millar Mikro-Tip, model MPC-500) with a pressure range of −50 to 300 mmHg and a sensitivity of 5 μV/V/mmHg.

2.3.2. Flow-meter measurements

Medi–Stim perivascular flow-meter probes were used, 8 and 12 mm in size, with a flow accuracy of 1%, resolution of 1 ml/min and flow sample rates were 333 Hz.

Left pulmonary artery flow was calculated as the difference between the ascending aorta and main pulmonary artery flow, after having ruled out intra-cardiac defects by color Doppler echocardiography and measurements of right atrium and main pulmonary artery O_2 saturations.

2.3.3. Tissue processing

Both lungs were fixed with 4% buffered formaldehyde by tracheal perfusion and immersion during 24 h. Up to 15 tissue samples for each lung were taken from the central (region of the anastomosis), post-central and peripheral (sub-pleural) areas of the vascular tree and embedded in paraffin. Histological slides were stained with hematoxylin–eosin as well as with a combination of van Gieson–elastin stain. All slides were examined and the vascular lesions graded according to the Heath–Edwards classification [23] by one of us (C.Y.G.), unaware of the origin of the specimens.

3. Results

As we already described in Section 2, the first pig died shortly after tracheal extubation because of pulmonary edema. Since then, all other animals have been treated with furosemide 1 mg/kg i.v. after opening the vascular clamps, dopamine 5 μg/kg i.v. until the end of the procedure and furosemide 40 mg/day/7 days per os, to prevent or reduce the negative consequences of the suddenly induced unilateral pulmonary edema. With this strategy we were able to extubate all other animals shortly after the end of the procedure and to send them all back to the farm within the same day.

The mean left-to-right shunt through the left pulmonary artery (left pulmonary artery flow/ascending aorta flow) diminished from 58.9 ± 9.6% immediately at the end of the surgical procedure to 4.5 ± 1.5% at the latest hemodynamic evaluation ($P < 0.01$), showing a significant increase of pulmonary vascular resistance in the left lung from a mean value of 19.3 Units at the end of the surgical procedure to a mean value of 232.0 Units. Pressures and oxygen saturations were identical in aorta and left pulmonary artery, confirming the presence of an unrestricted end-to-side anastomosis between descending thoracic aorta and left pulmonary artery.

The latest hemodynamic evaluations (Table 1) showed that while in the left hypertensive lung, despite 30 min exposure to $FiO_2 = 1.0$, the pulmonary vascular resistance failed to decrease with only a significant increase of the systolic pressure and pO_2, in the left pulmonary artery, the right lung had only a mild elevation of the arterial pressures and remained responsive to oxygen vasodilatation.

Lung histology showed normal morphology of pulmonary arteries in all the specimens harvested from the right lungs (Fig. 2).

Irreversible vascular lesions were present in all the left lungs, with intimal fibrosis and medial hypertrophy (mean Heath–Edwards score = 4.4) in specimen harvested after 6–9 weeks of exposure to high pressure, flow and oxygen saturation (Fig. 3), while vascular occlusions, plexiform and dilatation lesions (mean Heath–Edwards score = 5.6) were present in specimen harvested after 12 weeks of pulmonary hypertension (Fig. 4).

4. Discussion

A variety of congenital heart defects with intra-cardiac communication, left-to-right shunt and increased pulmonary blood flow may be complicated by the development of pulmonary hypertension. Prolonged pulmonary hypertension may result in irreversible vascular lesions, leading to pulmonary vascular obstructive disease and the corresponding clinical pattern of Eisenmenger syndrome [1–10].

The severity of the vascular remodeling in the pulmonary vessels appears in direct correlation with the increase of the

Table 1
Data obtained from latest hemodynamic evaluation

	Left pulmonary artery			Right pulmonary artery		
	$FiO_2 = 0.21$	$FiO_2 = 1.0$	*P* value	$FiO_2 = 0.21$	$FiO_2 = 1.0$	*P* value
Pressure (mmHg)						
Systolic	77.8 ± 13.1	97.0 ± 11.3	<0.05	29.2 ± 3.7	23.0 ± 2.2	<0.05
Diastolic	46.2 ± 13.4	54.0 ± 11.1	NS	17.8 ± 5.2	10.4 ± 3.4	<0.05
Mean	59.6 ± 9.5	72.6 ± 10.5	NS	23.4 ± 4.4	16.2 ± 2.8	<0.05
O_2 saturation (%)	97.2 ± 2.4	100 ± 0	NS	64.9 ± 2.1	77.9 ± 0.9	<0.005
pO_2 (kPa)	12.2 ± 4.1	60.0 ± 3.2	<0.001	3.9 ± 0.6	4.8 ± 0.3	<0.05

pulmonary blood flow, the pressure and oxygen saturation at which it is delivered, and the duration. Various experimental models of pulmonary hypertension have been proposed [11–22], but none of them with adequate and simultaneous increase of pulmonary blood flow, pressure and oxygen saturation in one lung, with the contra-lateral lung remaining as the control lung.

The model we devised consists of a reproducible experimental model with unilateral pulmonary hypertension (left lung perfused with elevated blood flow, systemic pressure and oxygen saturation), with the right lung remaining as the control lung, perfused with normal pressure and oxygen saturation with only increased blood flow.

The significant increase of pulmonary vascular resistance observed in the left lung after a period of exposure to systemic pressure and oxygen saturation, together with the concomitant irreversible vascular lesions, demonstrated at histological level, confirm that our model developed a clinico-pathological pattern of unilateral pulmonary vascular obstructive disease. The degree of gravity of the observed vascular lesions, as expected, was correlated with the duration of pulmonary hypertension.

Our model also confirmed that even a substantial increase of pulmonary blood flow in the contra-lateral lung, unaccompanied by increase in pressure and oxygen saturation, was unable to determine any change in the morphology of the pulmonary vessels, as well as in the hemodynamics. The only consequence to the increased pulmonary blood flow for the same duration was a mild elevation of the pulmonary artery pressures, with preserved reactive dilatation in response to inhaled oxygen.

Of course we are aware that our experimental model reproduces the vascular changes present in Eisenmenger syndrome, without the concomitant right ventricular hypertrophy, but our purpose was to address the changes in the pulmonary circulation, not on the myocardial function.

In conclusion, this experimental model of unilateral pulmonary hypertension reproduces the clinical and pathological entity corresponding to the pulmonary vascular obstructive disease developed in patients with Eisenmenger syndrome.

The model may serve well in the following series of studies:

- all investigations directed at the underlying mechanisms responsible for pulmonary vascular disease caused by increased blood flow, pressure and oxygen saturation;
- comparison between hypertensive versus normotensive lung in the same subject with regard to pathophysiology, gene expression as well as protein and product

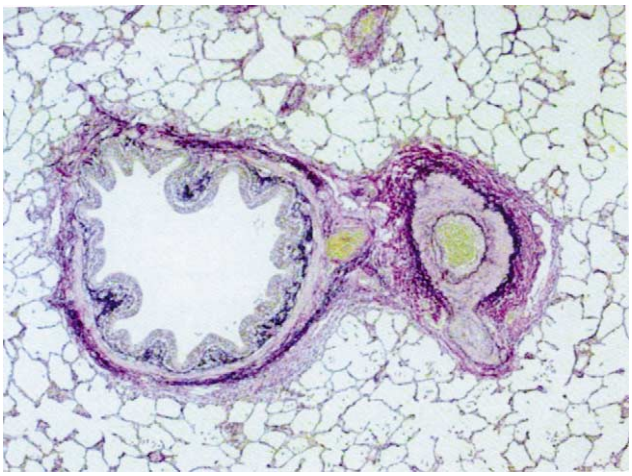

Fig. 3. Histology (van Gieson–elastin stain of the left lung after 9 weeks, with medial and intimal hypertrophy.

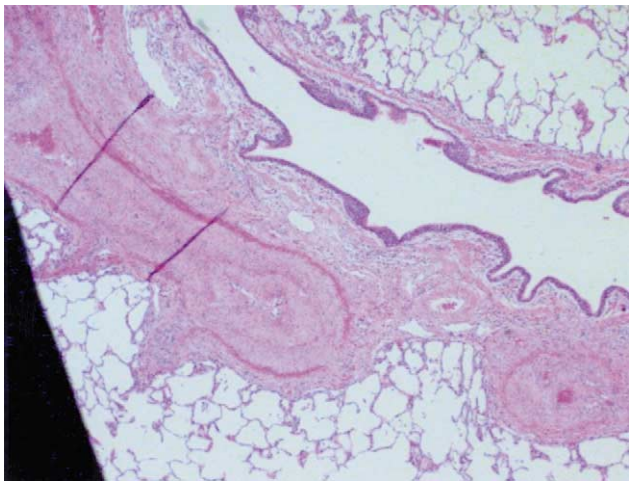

Fig. 4. Histology (van Gieson–elastin stain of the left lung after 12 weeks showing vascular occlusions, plexiform and dilatation lesions.

formation;
- test the effects of interventions commonly used clinically, such as inhaled nitric oxide, to treat pulmonary hypertension;
- test new drugs potentially active on pulmonary hypertension; and
- device new strategies aiming at slowing the progression of the pulmonary vascular obstructive disease.

Acknowledgements

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Appendix A. Conference discussion

Dr A. Haverich (*Hannover, Germany*): You know and I know that this model is not new. There was Greek groups in the late '60s who did subclavian artery to the upper lobe branch of the pulmonary artery with a similar effect on the histology.

I have two questions. One, what do you think would happen if you would reverse this anastomosis? And have you done these experiments with the question of spontaneous reversal of pulmonary hypertension?

And second, do you really think that this model mimics the Eisenmenger situation since the right ventricle is not affected by the disease?

Dr Corno: I'm aware of the shunt done with the left subclavian artery on the upper lobe of the pulmonary artery. There is also more recent work done in the United States, and published two years ago in the Journal of Thoracic and Cardiovascular Surgery, with just the left upper lobe involved. But in all the above studies, the problem is that there was no one contralateral lung available with normal perfusion as a control lung. This is what I consider an advantage of our model compared to the model you just quoted.

In regard to your question, yes, of course, we studied this model because, first of all, we wanted to validate the model. And, of course, we have in mind the strategies that maybe you have seen in the second slide when I talked about this procedure. So the plan is now, first of all, to recreate the same model, to reduce the pulmonary artery pressure with progressive banding into the left pulmonary artery, and to follow the hemodynamic and then histological lesion. The question is, I'm sure, then if the pulmonary artery pressure is going down when you band, because we've already done this acutely in two animals. And regarding the pressure, we were able to reduce the systolic pressure in the pulmonary artery to 50% and to 60% of the systemic. So we can lower the pulmonary artery pressure.

Is this followed by a regression of what they call "irreversible histological lesions"? At the moment it is just a dream, but we want to test this hypothesis.

Regarding the right ventricle, we are not interested in the right ventricle. If the right ventricle is able to work, sustaining increased pulmonary vascular resistance and systemic pulmonary artery pressure, once you are able to lower the pulmonary vascular resistance and pressure, the right ventricle should be able to work anyway.