Fatal persistent pulmonary hypertension presenting late in the neonatal period

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Abstract
Two cases of fatal idiopathic persistent pulmonary hypertension presented late in the neonatal period. Lungs were examined histologically by light and electron microscopy, and immunocytochemical studies were used to identify nerves. There was extension of medial smooth muscle distally along the arterial pathway so that most precapillary arteries had completely muscular walls, which in some cases completely obliterated the vessel lumen. Enlarged endothelial cells also contributed to the reduction in the size of the lumen. Nerve fibres accompanying muscular arteries were found in the alveolar region, more distal than is normal. The predominant neuropeptide was the vasoconstrictor tyrosine.

Possible aetiological factors in persistent pulmonary hypertension of the newborn are increased muscularity of the peripheral pulmonary arteries antenatally, an increase in the number of vasoconstrictor nerves, or an imbalance in the production of leukotrienes and prostacyclins in the perinatal period.

Persistent pulmonary hypertension of the newborn is a failure of the pulmonary circulation to adapt successfully to postnatal life. Associated with a wide variety of neonatal cardiopulmonary disorders, it is characterised by an inappropriately high pulmonary vascular resistance with subsequent right to left shunting of blood at the foramen ovale, the ductus arteriosus, the lungs, or a combination. In this paper we describe two fatal cases of idiopathic persistent pulmonary hypertension of the newborn in infants who presented late in the neonatal period. The structural changes found in the lungs were more severe and extensive than those previously described.

Case reports
CASE 1
A male infant weighing 3400 g was born at full term after a normal pregnancy and delivery. The mother had received carbamazepine for epilepsy. There was no relevant family history. Apgar scores were 7 at 1 minute and 10 at 5 minutes. At 2 days of age the baby had two cyanotic episodes and was diagnosed as having a respiratory tract infection, although no pathogens were isolated. He was re-admitted at the age of 20 days for investigation of failure to thrive. He had a further cyanotic episode, which was attributed to an Escherichia coli urinary tract infection. At 2 months of age it was noted that he became cyanosed when crying and feeding. He also had tachypnoea and sweating at rest. Arterial blood gas analysis while breathing air showed: pH 7.42, carbon dioxide tension 4.9 kPa, oxygen tension 4.7 kPa, and base excess 0.7. He was referred to the Brompton Hospital for investigation of suspected cyanotic heart disease.

On examination his weight had fallen to below the third centile for age. He was cyanosed and there was a prominent right ventricular impulse. The pulmonary component of the second sound was loud but there were no murmurs. His respiratory rate was 46 breaths/minute. The chest radiograph showed mild cardiomegaly and normal pulmonary vascular markings. The electrocardiogram showed a mean frontal QRS axis of +150° and right ventricular hypertrophy. The cross sectional echocardiogram showed normal anatomy of the heart with normal atrioventricular and ventriculoarterial connections. The right atrium was enlarged and the interatrial septum bowed from right to left. The right ventricle was grossly dilated with poor systolic function. The parasternal short axis view showed that the left ventricle was 'squashed', suggesting a high right ventricular pressure.

Cardiac catheterisation was carried out under general anaesthesia. The oxygen saturation in the aorta was 53%. The right ventricular pressure was 90/0.4 mm Hg with a simultaneous left ventricular pressure of 68/5 mm Hg. Using an estimated oxygen consumption of 150 ml/min/m² pulmonary vascular resistance was 24 units/m² in air and 10.5 units/m² in 100% oxygen.

Angiography showed poor right ventricular function but no intracardiac abnormalities. A diagnosis of persistent pulmonary hypertension of the newborn was made, and a pulmonary arterial line was left in.

The patient was extubated into headbox oxygen. The pulmonary artery pressure at this stage was 156/80 mm Hg with a systemic pressure of 80/30 mm Hg. Prostacyclin and adenosine were infused through the pulmonary arterial line. The patient was placed in a continuous subatmospheric (negative) extrathoracic pressure chamber and given 100% oxygen.

None of these manoeuvres succeeded in lowering the pulmonary artery pressure appreciably, and it remained higher than the systemic pressure. On the day after cardiac catheterisation the patient had a cardiorespiratory arrest, which did not respond to intubation and standard resuscitative measures. Consent for necropsy was obtained.
CASE 2
A male infant weighing 3500 g was born at full term after a normal pregnancy and delivery. There was no relevant family history. Apgar scores were 8 at 1 minute and 9 at 5 minutes. He was noted to be cyanosed and breathless at 10 days of age. Arterial blood gas while breathing air showed: pH 7.19, carbon dioxide tension 3-2 kPa, oxygen tension 7-0 kPa, and base excess -17-9. He was transferred to the Hospital for Sick Children for investigation of suspected cyanotic heart disease.

On examination his weight had dropped to below the third centile and he was cyanosed and tachypnoeic. He had a prominent right ventricular impulse and the pulmonary component of the second heart sound was accentuated. There were no murmurs. The chest radiograph showed a heart of normal size with normal pulmonary vascular markings. The electrocardiogram showed a mean QRS axis of +140° and right ventricular hypertrophy. Cross sectional echocardiography showed normal anatomy of the heart with normal atriocentric and ven- trículoartorial connections. The interatrial septum was bowed from right to left. The right heart chambers were dilated and there was a small duct of no haemodynamic importance.

Cardiac catheterisation carried out under local anaesthesia at 4 weeks of age confirmed the echocardiographic findings and showed a pulmonary arterial pressure of 77/28 mm Hg with a simultaneous systemic arterial pressure of 75/36 mm Hg. Using an estimated oxygen consumption of 150 ml/min/m² the pulmonary vascular resistance was 15-6 units/m² in air and 15-0 units/m² in 100% oxygen. A diagnosis of persistent pulmonary hypertension of the newborn was made. At 5 weeks of age a Ladd’s procedure for malrotation of the bowel was carried out, and the baby made an uneventful recovery. A week later he developed congestive cardiac failure and was treated with digoxin and frusemide.

A second cardiac catheterisation carried out at 2 months of age under general anaesthesia showed a saturation in the aorta of 78% and equal pulmonary artery and aortic pressures of 100/47 mm Hg. Pulmonary vascular resistance in air was 13-5 units/m² and in 100% oxygen 8-7 units/m². During cardiac catheterisation nifedipine, aminophylline, and isoprenaline all failed to reduce either the pulmonary arterial pressure or resistance. A pulmonary arterial line was left in. Prostacyclin was infused overnight through the pulmonary arterial line and continuous monitoring showed a slight reduction in pulmonary pressure to just below the systemic pressure. The baby continued to deteriorate, however, and hydralazine, tolazoline, further infusions of isoprenaline, and increased doses of prostacyclin had no effect. He died at 2-5 months of age. Consent for necropsy was obtained.

Histopathological findings
MACROSCOPIC FINDINGS
In both cases the right atrium and the tricuspid valve ring were dilated and the right ventricle was hypertrophied. Case 1 had subendocardial calcific streaks in the right anterior papillary muscle. The pulmonary trunk and central pulmonary arteries were dilated. The ductus arteriosus was closed in case 1 and persistent in case 2. The foramen ovale was patent in both cases, and in case 1 was membrane like with multiple perforations. The lungs looked normal, but mucopus was present in the large airways of case 2.

MICROSCOPIC STUDIES
Blocks of lung tissue were fixed in formol saline after intrabronchial inflation for light microscopic morphological studies, in a modified Bouin’s solution for immunocytochemical examination of the innervation, or glutaraldehyde for electron microscopic studies. In each case at least five blocks of tissue were taken from different parts of the lung for microscopic examination. Morphometric analysis included measurements of percentage medial thickness of respiratory unit arteries and veins, of the size and structure of vessels accompanying identifiable airways, and of the number of small vessels in the alveolar region.

Findings were similar in the two cases. Airway structure was normal. A normal number of alveoli had developed but their walls were thicker than normal as a result of an increase in the number and size of capillaries rather than an increase in the amount of connective tissue. The connective tissue septa and the collagenous sheets around the airways and blood vessels were prominent. The preacinar and intra-acinar pulmonary arteries had grossly thickened walls caused by an increase in vascular smooth muscle. Mean percentage medial thickness of arteries 51-100 μm external diameter was 36-2% in case 1 and 39-2% in case 2 (normal 11-6%). Muscle was increased in arteries of all size ranges and there was a thick muscle coat in small precapillary vessels lying in alveolar walls, which are normally not muscular in structure (fig 1). The muscle was so thick that it almost obliterated.
the lumen in many arteries. Further reduction in lumen size was the result of enlarged endothelial cells, which were chunky in outline. In some of the small vessels these cells totally occluded the lumen (fig 2). Mild cellular intimal proliferation was occasionally seen in the small muscular arteries proximal to the respiratory unit. This was cellular, loosely organised, and not obstructing the artery.

Pulmonary arteries in both the preacinar and acinar regions had normal external diameters in case 1 and increased diameters in case 2 because of the thick media. The number of small pulmonary arteries related to the number of alveoli were normal for age. The thickness of the walls of the pulmonary veins was increased. In veins that were 51-100 μm in external diameter the percentage wall thickness was 11.7% in case 1 and 7.5% in case 2 (normal 4.7%).

There were enlarged veins and lymphatics close to pulmonary arteries and airways in the intra-acinar region (fig 1).

Immunohistochemical studies using the general neuronal marker protein gene product 9-5 showed nerve fibres in the adventitia and primarily at the adventitial-medial border in all muscular arteries as far as the alveolar region. The neuropeptide tyrosine, a vasoconstrictive agent, was the predominant vasoactive peptide in these nerves (fig 3).

Discussion

The absence of severe cyanosis at birth in these infants suggests that any right to left shunt must have been small and that pulmonary arterial pressure may have fallen, though probably not to normal. Cyanosis became apparent later as pulmonary arterial pressure rose and a right to left atrial shunt developed, the arterial duct having closed by the time of death of the first patient and being small in the second. The cyanosis may also have been partly the result of intrapulmonary shunting. This contrasts with the usual presentation of persistent pulmonary hypertension of the newborn in babies born at full term who characteristically present with severe cyanosis and hypoxaemia at birth.

The histopathological appearances were severe both in absolute terms and in relation to the age of the infants. The lungs were of normal size, with normal airway and alveolar development. In the normal newborn lung the percentage medial thickness of arteries less than 250 μm in diameter decreases rapidly during the first three days of life to approach the normal adult 'mature' medial thickness. This reduction in thickness is thought to be responsible for the rapid fall in resistance that occurs at birth.'
and is associated with a thinning of the endothelial cells. In the present cases it is probable that peripheral pulmonary arteries remained thick walled after birth in both infants and so pulmonary vascular resistance did not fall normally.

Examination of the structure of the arteries running along the peripheral airways showed extension of muscle distally along the arterial pathway so that most precapillary arteries had a completely muscular wall. In normal lungs at birth there are no muscular arteries beyond the level of the alveolar ducts and even in the adult most of the arteries in the alveolar region are non-muscular. Though there was no obvious reduction in the number of peripheral blood vessels, the increase in muscle cells and enlarged endothelial cells led to a reduced luminal cross-sectional area. The thick enlarged appearance of the endothelial cells suggested that these vessels may not have been recruited into the pulmonary circulation at birth as they did not show the normal adaptive changes. There was premature extension of the nerves to the periphery associated with the increase in muscle. In normal infants nerves do not extend far beyond the respiratory bronchiolar region. These nerves were predominantly vasoconstrictive, and could have led to an increase in resistance.

None of the aetiological factors usually implicated in the pathogenesis of persistent pulmonary hypertension of the newborn (for example, birth asphyxia, polythemia, and hypoglycaemia) was present. An increase in the muscularity of the small pulmonary arteries has been reported in cases where the ductus arteriosus has closed prematurely thereby increasing pulmonary artery pressure antenatally; this possibility could be implicated in case 1 but not in case 2 in which there was a small persistent ductus arteriosus. The magnitude of the pulmonary vascular change and the thickness of the walls of the intra-acinar arteries (which in these cases exceeded the normal fetal amounts) suggests that at least some of the increased muscle developed before birth. This might have happened if the pulmonary circulation of these infants was abnormally sensitive to the hypoxic environment of fetal life, responding by excessive vasoconstriction and then by hypertrophy of muscle. Certainly after birth sensitivity to hypoxia varies considerably among individual infants.

Alternatively, the increased muscularity may have represented a chance exuberant growth of normal muscle within the pulmonary circulation which, after birth, initiated a vicious circle to maintain arterial pressure leading to further hypertrophy of the arterial muscle. In the presence of pulmonary hypertension pericytes can rapidly differentiate into muscle cells, and in experimental hypoxia in rats and pigs do so within three days of exposure. It may be therefore that the fundamental abnormality in the infants described is the excessive muscularity antenatally of arteries less than 250 μm in diameter. Alternatively, the condition may have a pharmacological or neurological aetiology. The phospholipid leukotriene LTD4 seems to act as a pulmonary vasoconstrictor in newborn lambs, and prostacyclin is a potent dilator of human fetal pulmonary arteries. The production of leukotriene falls while that of prostacyclin rises in the perinatal period. Endothelial vasoconstrictors such as platelet derived growth factor are released during fetal life and stop being produced after birth. Studies in porcine and human lungs have shown that sympathetic nerves contain vasoconstrictor neurotransmitters. In man the density of nerve fibres is low in the arteries of the respiratory unit at birth but may increase during childhood. These nerves are predominantly vasoconstrictor in effect and an increase in the number, distribution, or function of these vasoconstrictor nerves may cause excessive pulmonary vasoconstriction. This may be responsible for the persistent pulmonary hypertension in these cases as there was extensive innervation in the small muscular peripheral arteries. The premature innervation of the muscularised peripheral arteries may be primary or secondary. Nerves can have a trophic affect on muscle cells, but increased muscularity may lead to increased innervation. There is no evidence of a link between maternal ingestion of carbamazepine (case 1) and the development of persistent pulmonary hypertension of the newborn.

Whatever the aetiology, the effect of a reduction in the diameter of the lumen of patent arteries, together with an increase in thickness of the walls, was to increase resistance by decreasing the cross sectional area of the pulmonary vascular bed. An increase in both the thickness of the muscle coat and the length of the muscularised pathway also increased the capacity to vasoconstrict. Even the smallest precapillary arteries had muscle cells in their walls and would be able to have constricted. Thus vasoconstriction seems to be an important mechanism leading to persistent pulmonary hypertension of the newborn.

The cases described illustrate the extreme pathological changes that may develop in persistent pulmonary hypertension of the newborn. The association between structure and function in neonatal pulmonary hypertension is complex and unclear. Studies of the association between vascular smooth muscle maturation, contractility, and innervation will— we hope— help to determine the aetiology and subsequently the treatment of this disease.

We thank Dr J Wharton for figure 3.

7 Hall SM, Haworth SG. Normal adaptation of pulmonary arterial innervation.