Effect of prostaglandin E₁ on pulmonary circulation in pulmonary atresia

A quantitative morphometric study

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SUMMARY The structural effect of prostaglandin E₁ on the pulmonary circulation in pulmonary atresia has been studied by applying quantitative morphometric techniques to the injected and inflated lungs of eight babies who had received prostaglandin E₁ for between 30 hours and 12 days.

The most striking effect was on the pulmonary arterial smooth muscle. Relative arterial medial thickness was reduced and muscle did not extend as far along the arterial pathway as compared with the normal and with untreated cases of pulmonary atresia, dying at a similar age. The reduction in muscularity tended to increase the longer the duration of infusion. In all cases the thin arterial media was less compact than normal, and localised aneurysmal dilatations occurred, varying in extent and severity between cases. The preacinar arteries were dilated in comparison with the untreated cases, but, by contrast, the intra-acinar arteries remained abnormally small. The number of intra-acinar arteries per unit area of lung was greater in prostaglandin E₁ treated than in untreated cases.

Infusion of prostaglandin E₁ is now the ideal emergency treatment for pulmonary atresia, but the findings in the present study suggest that it should be given for as short a time as possible before the pulmonary blood flow is increased by surgical treatment.

Prostaglandin E₁ (PGE₁) dilates the ductus arteriosus¹ and is therefore used in the emergency treatment of newborn infants with pulmonary atresia.² The effect of PGE₁ on the lungs of such patients is unknown. Acute experiments on healthy fetal and newborn goats,^{3 4} and in the adult animal in a variety of species,⁵⁻⁷ demonstrate a fall in pulmonary vascular resistance. In the human adult infusion on PGE₁ lowers pulmonary vascular resistance, considerably in the presence of pulmonary hypertension, but only slightly in the normal lung.^{8 9}

A quantitative morphometric study of the lungs of untreated children dying with pulmonary atresia showed a reduction in pulmonary arterial muscle, pre- and intra-acinar arterial size, and intra-acinar arterial number,¹⁰ compared with normal.^{11 12} In the present study the effect of PGE₁ on the lung vessels of patients with pulmonary atresia, dying at a similar age, has been analysed using the same techniques.

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Subjects

The lungs of eight patients with pulmonary atresia were examined (Table 1). All children underwent surgical treatment. In four children an aorto-pulmonary anastomosis was performed, in three a Brock procedure, one of whom also had formalin infiltration of the ductus arteriosus, and, in the remaining case, the right ventricular outflow tract was reconstructed. PGE₁ was given preoperatively in all cases and continued after operation in cases 3 and 8. The dose of PGE₁ given varied between 0.01 and 0.1 µg/kg per min, the majority of patients receiving at least 0.08 µg/kg per min for most of the time.

Preoperative infusion of PGE₁ produced a clinical improvement in all cases. The increase in systemic arterial oxygen tension varied between 8 and 20 mmHg (mean 13 mmHg). Five children survived surgery, but only case 5 appeared to have had a significant increase in pulmonary blood flow. He

Table 1 Clinical data

Case no.	Age PGE ₁ started (h)	Duration (h)	Dose (µg/kg per min)	Route	Time interval between end of PGE ₁ infusion and death (d)	Age at death (d)
1	14	30	0.04-0.08	Aorta	0	2
2	10	72	0.05-0.10	Peripheral vein	0	4
3	24	74	0.01-0.12	Umbilical vein	0	5
4	12	6 d	0.02-0.10	Umbilical and peripheral vein	4	23
5	24	12 d	0.01-0.05	Aorta	3	16
6	41	15	0.02-0.10	Umbilical and peripheral vein	13	15
7	8 d	8	0.1	Aorta	8 h	9
8	40 d	29	0.01-0.10	Peripheral vein	7	50

died three days after operation with disseminated intravascular coagulation.

The structural findings are compared with those in a published series, consisting of six 'untreated' cases of pulmonary atresia who did not receive PGE₁ and died without effective surgical treatment at between 1 and 28 days of age.¹⁰

Methods

The pulmonary arteries to both lungs were injected in all, save case 7, where the right pulmonary artery and left pulmonary veins were injected. A Micropaque gelatin suspension at 60°C was injected at a pressure of 100 cm of water: this injection technique distends and fills all vessels larger than 15 µm in diameter. The lungs were then inflated with a buffered formol saline solution at a pressure of 45 cm of water and allowed to fix. Lung volume was determined by water displacement, and each lung radiographed and sliced,^{13 14} blocks of tissue being selected for microscopical examination by a random sampling technique.¹⁵

QUANTITATIVE ANALYSIS OF STRUCTURAL FEATURES OF LUNG

From the arteriogram the following features were assessed: hilar and intrapulmonary pattern of branching and the density of the background haze which reflects the number of small peripheral arteries¹⁶; lumen diameter of the posterior basal arterial pathway measured at the hilum and at 25 per cent intervals from the hilum to the pleural surface.

Microscopically, the following features of peripheral arterial structure were studied: (1) The external diameter and medial thickness of an individual artery was measured and the mean percentage medial thickness calculated. (2) The structure of the arterial wall, whether muscular, partially muscular, or non-muscular was related to size in the large population of vessels. (3) Where a small artery accompanies a small airway it is characterised by reference to the type of airway it

accompanies. Thus the structure of an artery could be related to airway level and in this way extension of muscle along the arterial pathway was established. (4) The number of arteries and alveoli were counted in the same area of lung section and the result expressed as a ratio: this made allowance for any difference in the degree of inflation in various lungs. (5) The proportion of lung volume occupied by various structures was established by a microscopical point-counting technique.¹⁵

Results

In all cases lung volume was within normal limits. Arteriography showed a normal arterial branching pattern in all cases (Fig. 1). Aneurysmal dilatation of the distal half, the muscular portion of the arterial pathway, occurred in all cases, but was pronounced in four (cases 4, 5, 6, and 7) (Fig. 1). In these four cases, between three and seven segments were involved in each lung.

GENERAL HISTOLOGICAL FEATURES

In all cases the arteries accompanying small bronchi and bronchioli were larger than normal relative to the size of the airway and appeared tortuous. Tortuosity extended from at least the level of the small bronchi down the arterial pathway to respiratory bronchiolar level, involving muscular, partially muscular, and non-muscular vessels. Dissecting aneurysms were seen in four cases, involving the precinar* muscular arteries. The arteries also appeared abnormally thin walled. The elastic fibres and muscle cells were arranged in a less compact fashion than normal, particularly the outermost elastic lamina which frequently peeled away from the vessel at some point around the circumference. Oedema produced small clear spaces, which were variable in size and frequently separated the medial components. These spaces showed no eosinophilia.

Dilatation of the lymphatic channels was a striking feature in cases 1 to 4. In these four cases the subpleural lymphatic channels, those in the

*An acinus is all the lung tissue distal to a terminal bronchiolus.

connective tissue septa within the lung and those in the perivascular sheath were abnormally distended. Lymphatic dilatation was present but less distinct in cases 5 and 6. In all cases the veins also appeared unduly prominent, appearing larger and more circular than is normal. Vein wall structure appeared normal.

ARTERIAL SIZE

On the postmortem arteriogram the lumen diameter of the preacinar arteries was generally greater than that of untreated patients dying with pulmonary atresia and similar to that seen in the normal child of similar age (Fig. 2). The difference in size between untreated and PGE_1 treated cases of pulmonary atresia was pronounced along the proximal half of the pathway, which consists of elastic arteries. Further along the pathway, however, microscopical examination revealed conspicuous dilatation of the

preacinar muscular vessels.

By contrast, within the acinus microscopical measurement showed a reduction in external diameter in seven cases (Table 2). At nearly all levels along the arterial pathway the arteries were smaller, even at 15 days, than is seen in the normal lung at three days, and were similar in size to those of untreated patients. In the remaining case (case 8) the findings were difficult to interpret because there were no normal or untreated cases of pulmonary atresia of similar age with which to make a comparison.

NUMBER OF INTRA-ACINAR ARTERIES

In all cases treated with PGE_1 , the arterial volume proportion was greater than in untreated cases, and was even greater than normal. The arterial volume proportion varied between 6.9 and 9.4 per cent in PGE_1 treated cases as compared with 1.5 to 3.9 per

Fig. 1 Postmortem arteriograms of the right lung: (a) normal aged 5 days (magnification $\times 1.1$); (b) PGE_1 treated, case 4, showing an increase in density of the background haze (magnification $\times 1.4$); (c) detail of (b) showing dilatations of pulmonary arteries (original magnification $\times 4.2$).

Fig. 2 Measurements from postmortem arteriograms: lumen diameter (mm) at 25 per cent intervals along the axial pathway from the hilum to the pleural surface is generally greater in PGE₁ treated than in untreated cases of pulmonary atresia. Interrupted line—mean of five untreated cases of pulmonary atresia (one to 15 days); solid lines—PGE₁ treated cases of pulmonary atresia (two to 23 days).

cent in the untreated, and 3.75 per cent in the normal cases. An increase in microscopical arterial volume indicates an increase in the size or number of intra-acinar arteries, or both, but in the present instance suggested an increase in arterial number. In all these cases the postmortem arteriogram showed an increase in density of the background haze, in comparison both with the normal and with the untreated cases of pulmonary atresia (Fig. 1). This observation also suggests an increase in arterial number, confirmed microscopically by finding a reduction in the alveolar/arterial ratio. The ratio varied between 8.6 and 10.69, lower than that of the normal of similar age, 18.9 to 20.3. In untreated pulmonary atresia, peripheral arterial number is reduced, giving an abnormally high alveolar/arterial ratio.

ARTERIAL MUSCULARITY

Percentage arterial medial thickness

In the normal newborn, both human and animal, the postnatal reduction in relative medial wall thickness in arteries smaller than 250 μ m in diameter occurs more rapidly than in larger vessels.^{12 17} In the present study, therefore, the

relative wall thickness in arteries below and above 250 μ m diameter was considered separately. In all eight cases relative medial thickness of arteries less than 250 μ m was below normal and even below that seen in untreated cases of pulmonary atresia (Fig. 3). In arteries larger than 250 μ m, in four cases (cases 1, 2, 3, and 8) muscularity was similar to that seen in untreated cases and below the normal for age. In the remaining four cases muscularity was less than that seen in untreated cases and even less than that seen in the normal adult (Fig. 4). Two of the three cases showing the greatest reduction in muscularity in arteries of all size groups also showed the most severe and widespread picture of aneurysmal dilatations on the postmortem arteriogram.

Extension of muscle along the arterial pathway

Examination of the structure of arteries in relation both to their size and to their position along the arterial pathway showed that in seven cases of pulmonary atresia given PGE₁ muscle did not extend as far to the periphery as in either untreated cases of pulmonary atresia or in normal children of similar age. The smaller arteries contained less muscle. The size of the smallest arteries entirely surrounded by a muscle coat was increased and larger arteries had a partially rather than an entirely muscular coat (Table 3). Examination of the structure of arteries accompanying the peripheral airways showed that at terminal bronchiolar level a greater proportion of arteries had a partially muscular rather than an entirely muscular wall (Table 4). More peripherally, at respiratory bronchiolar and alveolar duct level, more arteries had a muscular rather than a partially muscular wall structure. In case 8, the oldest child dying at

Table 2 Size of arteries accompanying peripheral airways

<i>External diameter (μm)</i>					
<i>Case no.</i>	<i>Age at death (d)</i>	<i>Alveolar wall</i>	<i>Alveolar duct</i>	<i>Respiratory bronchiolus</i>	<i>Terminal bronchiolus</i>
1	2	27.2	42.3	63.8	107.1
2	4	22.8	34.5	76.1	112.0
3	5	22.4	35.4	57.0	98.0
4	23	22.4	42.7	77.8	109.2
5	16	25.2	52.9	85.8	117.0
6	15	28.0	45.8	75.6	149.3
7	9	27.1	44.0	70.5	100.8
8	50	32.8	51.7	123.7	147.2
Untreated pulmonary atresia (n=5)					
	1 to 15	32.5 to 61.0		68.2 to 95.0	104 to 143
Normal ¹²					
	3 d to 4 mth	32.1 to 33.0	65.0 to 86.5	92.7 to 122.3	118 to 197

Fig. 3 Percentage arterial medial thickness related to external diameter (μm), showing the reduction in muscularity in cases of pulmonary atresia given PGE_1 , as compared with untreated and normal cases. $\bullet$ — $\bullet$ normal three days; $\bullet$ — $\bullet$ Mean of five untreated cases of pulmonary atresia. Solid lines represent PGE_1 treated, numbered cases, of similar age.

50 days, the distribution of muscle along the arterial pathway was normal.

SUMMARY OF PATHOLOGICAL FINDINGS

Comparison of the PGE_1 treated with the untreated cases of pulmonary atresia and with the normal showed the following: the preacinar arteries were dilated and tortuous, but the intra-acinar arteries remained abnormally small, similar in size to those of untreated cases. The number of intra-acinar arteries per unit area of lung was increased in relation to untreated cases and even exceeded the number found in the normal newborn lung. The lymphatic channels were abnormally distended in four cases. The walls of the muscular and partially muscular arteries appeared oedematous, the media was less compact than normal, and the outer elastic lamina was frequently broken. In the large muscular arteries localised aneurysmal dilatations occurred and dissecting aneurysms were present microscopically in four cases. Pulmonary arterial smooth muscle was reduced. Percentage arterial medial thickness was reduced, and muscle did not extend as far along the arterial pathway as in either the normal or untreated cases of pulmonary atresia.

Discussion

In this series of newborn children with pulmonary atresia treated with PGE_1 , the most striking abnormality was a reduction in pulmonary arterial smooth muscle. This feature cannot be attributed

to the increase in blood flow achieved by dilating the ductus arteriosus. In congenital heart disease an abnormally high flow is associated with increased pulmonary arterial muscle, even when there is little increase in pressure,²⁰ and in pulmonary atresia or stenosis an aortopulmonary anastomosis is frequently associated with increased muscularity.^{10 21}

The morphological findings in the present study are consistent with the physiological and in vitro studies on the action of PGE_1 on the pulmonary vasculature. PGE_1 relaxes isolated strips of pulmonary vascular smooth muscle, taken from both the mature and fetal animal.^{22 23} In acute human, dog, swine, and sheep studies, infusion of PGE_1 reduces pulmonary vascular resistance, though the magnitude of the response is species dependent.^{6 7} Holding the pulmonary blood flow constant, PGE_1 lowered the resistance by 51.1 per cent in the fetal goat, a response attributed to pulmonary vasodilatation.³ The findings of an increased number of injection filled intra-acinar arteries in the present study suggests that recruitment of peripheral arteries may be at least as important as dilatation in helping to reduce pulmonary vascular resistance.²⁴

This is the first study to show that prolonged infusion of PGE_1 can, at least in the newborn human lung, produce a reduction in pulmonary arterial smooth muscle. This finding suggests that the effect of PGE_1 on the smooth muscle cells in acute studies is manifested as a structural change after prolonged infusion.

The effects of PGE_1 on the structural properties of the smooth muscle cell are not understood, but PGE_1 is known to change the differentiating state of human skin and lung fibroblasts.²⁵ These cells respond to PGE_1 by a reduction in collagen synthesis. The response is rapid, a 47 per cent reduction in collagen synthesis appearing after only six hours of exposure to PGE_1 . As with systemic and pulmonary arterial vascular smooth muscle cells, fibroblasts exposed to PGE_1 show an increase in cyclic nucleotides.^{23 26 27}

In the present study the smooth muscle cells probably became less specialised. Meyrick and Reid²⁸ showed that the partially muscular and non-muscular regions of the arterial pathway contain intermediate cells and pericytes. These cells contain fewer contractile filaments than a smooth muscle cell and lie internal to a single elastic lamina. When rats are exposed to hypoxia these cells differentiate to form new muscle.^{28 29} Recovery is associated with a reduction in muscularity, and arteries entirely surrounded by muscle become partially muscular and the partially muscular become non-muscular vessels³⁰; these findings are similar to those in children with pulmonary atresia given PGE_1 .

Prostaglandin E₁ was given to five children during the first 24 hours of life. Human and animal studies on the normal lung show that it is during the first 24 hours of life that lung structure is changing rapidly and adapting to extrauterine

life.^{12 17} All the children given PGE₁ showed a greater reduction in muscularity in arteries less than 250 μ m in diameter than in either normal or untreated cases. This suggests that PGE₁, given at a time when the pulmonary circulation is probably

Fig. 4 Photomicrographs of two peripheral pulmonary arteries of identical size from (a) an untreated case of pulmonary atresia and (b) a case of pulmonary atresia given PGE₁ for six days, showing the difference in thickness of the media between the arrows. (Original magnification $\times 414$.)

most susceptible to change, exaggerated the normal process of adaptation to extrauterine life. It is interesting to observe that in the normal child the plasma prostaglandin E concentrations are higher during the first 24 hours than at any other time of life, falling rapidly by the second and third day.³¹

By contrast, Manchester *et al.*³² reported two infants born of mothers given the prostaglandin synthetase inhibitor, indomethacin, who experienced persistence of the fetal circulation, attributed to an abnormally high pulmonary vascular resistance, in the absence of a cardiac malformation or parenchymal lung disease. Indomethacin treated neonatal goats show an exaggerated response to hypoxia.³³ Levin *et al.*³⁴ reported an increase in pulmonary arterial medial width/external diameter ratio in two children born of mothers who took either salicylates or indomethacin during pregnancy. In one patient the ductus arteriosus remained patent, suggesting a direct effect of prostaglandin synthetase inhibitor on the pulmonary vascular smooth muscle, rather than the increased muscularity being secondary to premature closure of the ductus arteriosus.

Infusion of PGE₁ appeared to weaken the walls of muscular pulmonary arteries. This did not occur in untreated cases of pulmonary atresia, nor in a 15-day-old baby dying several days after a successful Waterston-Cooley anastomosis had been performed, but who had not received PGE₁.¹⁰

Infusion of PGE₁ also appeared to have a weakening effect on the wall of the ductus arteriosus in six of the seven cases in the present series in which the ductus was examined (A C Gittenberger-de Groot, 1979, personal communication). In four other babies there was 'oedema of the media with separation of medial components by clear spaces, pathological interruption of the internal elastic lamina and intimal laceration' after PGE₁ infusion,³⁵ changes similar to those found in the pulmonary arteries of the present series.

Table 3 Arterial structural type related to external diameter

Case no.	Age at death (d)	Largest non-muscular (µm)	Range of partially muscular (µm)	Smallest muscular (µm)
1	2	95.2	58.8-161	175
2	4	120.4	58.8-224	196
3	5	133.0	39.2-196	117.6
4	23	123.2	28.0-259	252
5	16	117.6	56.0-308	252
6	15	100.8	72.8-224	245
7	9	128.8	53.2-189	154
8	50	98.0	56.0-151.2	140
Normal ¹⁸				
	3	152.0	40.0-180	120
	4 mth	130.0	40.0-170	175
	10 mth	125.0	55.0-220	112

Table 4 Arterial structural type related to peripheral airway level

Airway level	Percentage of each type of vessel		
	Normal	Pulmonary atresia untreated	Pulmonary atresia given PGE ₁
<i>Terminal bronchioli</i>			
Non-muscular artery	9.7	3.7	8.3
Partially muscular artery	66.3	88.0	91.7
Muscular artery	24.0	8.3	0
<i>Respiratory bronchioli</i>			
Non-muscular artery	43.0	41.2	56.6
Partially muscular artery	57.0	56.3	43.4
Muscular	0	2.5	0
<i>Alveolar ducts</i>			
Non-muscular artery	88.0	76.0	92.9
Partially muscular artery	12.0	24.0	7.1
Muscular artery	0	0	0
No. of cases	3*	7	7 (cases 1-7)

* Hislop.¹⁸

Microscopical examination showed widespread distension of the lymphatic channels in several cases. PGE₁ increases the permeability of small blood vessels in the skin, doing so at a dose as low as 50 ng, and in cremaster muscle.³⁶ Studies on the sensitised guinea-pig lung showed that release of PGE₁ from the lung was associated with leakage of fluid.³⁷ If PGE₁ does, indeed, increase the permeability of the small pulmonary vessels there are important clinical implications. In the normal lung, the rate of lymph clearance is higher during the first hours of life than at any other time as fetal lung fluid is cleared.³⁸ In pulmonary atresia, therefore, PGE₁ treatment would be better started after this time, if at all possible.

The present series of cases is too small to show whether or not there is a clear relation between dose, duration, and structural change. There was, however, a progressive reduction in muscularity the greater the duration of infusion in children started on PGE₁ during the first 24 hours of life (Fig. 3). The two cases receiving PGE₁ for the longest period of time had the most severe and widespread aneurysmal dilatation of muscular pulmonary arteries. In addition, children given PGE₁ for a similar length of time showed a similar structural response.

Several babies survived for some time after the PGE₁ infusion was stopped, raising the possibility that the structural changes seen at necropsy might not entirely represent the action of the drug. The structural findings in these babies did not, however, differ in kind from those found in infants dying while being infused with the drug. The most severe change

occurred in three cases dying at between three and 13 days after stopping the infusion, suggesting that whatever change had been induced by the PGE₁ had either persisted or, indeed, had progressed after stopping the drug. The pulmonary blood flow probably never increased sufficiently in any patient to a point at which stimulation of vascular smooth muscle development might have occurred.

Case 8 showed less structural change than the other seven cases. This child might, however, have had a higher pulmonary blood flow and thus more pulmonary arterial muscle initially because treatment was not required until 40 days of age. At necropsy, the ductus arteriosus was relatively large and had the structural characteristics of a persistent ductus arteriosus rather than those of a normally closing ductus, as found in the six out of seven other cases examined (A C Gittenberger-de Groot, 1979, personal communication). In addition, starting treatment with PGE₁ at 40 days might produce less structural change than when started within the first hours of life.

CLINICAL IMPLICATIONS

Infusion of PGE₁ in babies with pulmonary atresia maintains the patency of the ductus arteriosus and ensures perfusion of the lung. In the present series, PGE₁ infusion was associated with dilatation of the precapillary arteries, a reduction in arterial muscularity, and an increase in number of intra-acinar pulmonary arteries. These effects are beneficial, since they are compatible with a reduction in pulmonary vascular resistance. Unfortunately, however, PGE₁ appears to have a weakening effect on the wall of muscular pulmonary arteries, an effect that may have been accentuated in these cases since pulmonary atresia is normally associated with a reduction in muscularity. Furthermore, the PGE₁ was generally used soon after birth when the pulmonary circulation is adapting most rapidly to extrauterine life, and, since adaptation is normally associated with a reduction in muscularity, the lung may have been particularly vulnerable to PGE₁ at this time. The pathological findings suggest that increased vascular permeability might be a second disquieting feature in the newborn infant.

The extent to which the structural changes in the muscular pulmonary arteries are reversible is unknown. Muscularity will, however, probably increase to a normal level given an increase in pulmonary arterial pressure and flow, just as muscularity increases abnormally in the presence of pulmonary hypertension.

Thus, the structural findings in the pulmonary circulation of children with pulmonary atresia treated with PGE₁ suggest that the drug is best

started after the first few hours of life and given until the arterial oxygen tension is increased and the child's condition has improved sufficiently for him to undergo surgical treatment.

The reduction in arterial muscularity seen in the present series suggests that PGE₁ may have a wider application in the newborn period. In particular, it may be useful in the management of patients with a normal heart in whom pulmonary vascular resistance remains raised after birth and in children with congenital heart disease who are born with abnormally thick-walled muscular pulmonary arteries.

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