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Pulmonary Flow Reserve in Children with Idiopathic Pulmonary Arterial Hypertension: Implications for Diagnosis and Therapy

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Abstract
Aims: Endothelial dysfunction is likely to contribute to the pathogenesis of idiopathic Pulmonary Arterial Hypertension (iPAH). We hypothesize that there are different patterns of endothelial cell function, which we studied in 17 children with iPAH.
Methods and Results: Pulmonary flow reserve was determined by acetylcholine infusion into segmental pulmonary arteries utilizing quantitative angiography and intra-arterial Doppler flow wire. Depending on the reactivity of the pulmonary to systemic arterial pressure ratio to short-term oxygen and intravenous epoprostenol or aerosolized iloprost responders and nonresponders were classified.

In 7 responders to oxygen-prostanoid administration the pressure ratio decreased from 0.9 ± 0.2 to 0.31 ± 0.11 ($p = 0.01$), the mean pulmonary flow reserve showed an excessive increase to 3.6 ± 2.0 ($p = 0.01$) after infusion of acetylcholine. In 10 non-responders the pressure ratios remained unchanged during oxygen-prostanoid testing. 4 of 5 patients without any effect to acetylcholine died despite long-term epoprostenol treatment. The other 5 nonresponders to oxygen-prostanoid showed an impaired but significant increase of the pulmonary flow reserve of 1.6 ± 1.1 ($p = 0.01$). 2 of these patients did not only improve clinically, but regained vascular reactivity by additional therapy with sildenafil.
Conclusion: Endothelial reactivity in iPAH is either extensive, impaired or absent. Acetylcholine infusion casts a light on the pathogenesis and has implications for therapy.

Key words: endothelium; acetylcholine; pulmonary hypertension; children

Introduction

Idiopathic Pulmonary Arterial Hypertension is usually a rapidly progressive disease of the pulmonary vasculature leading to right heart failure and death. Three factors are thought to cause an increase of the pulmonary vascular resistance: vasoconstriction, remodeling of the pulmonary vessel wall, and in situ thrombosis [1]. A substantial number of mechanisms have been implicated in the pathogenesis of pulmonary arterial hypertension, as recently reviewed by Humbert et al. [2]. Impaired chronic production of vasodilators seems responsible for the increase in vascular tone and perhaps also for the vascular remodeling. In lungs of patients with pulmonary hypertension a reduced expression of endothelial nitric oxide synthase [3] as well as prostacyclin synthase expression [4] has been found, along with prolonged overexpression of vasoconstrictors such as endothelin [5]. Consequently, nitric oxide and prostacyclin currently represent logical pharmacologic targets or are being used therapeutically. In fact the treatment of iPAH has improved survival compared with historical reports before the use of calcium channel blocker (CCB) and/or epoprostenol [6]. However, the current therapy is still not satisfactory, and far from providing a cure. Therapeutic strategies in iPAH depend on the outcome of acute vasodilator testing [6, 7]. We favour the stringent criteria for acute vasoreactivity that were recently defined by Sitbon et al. [7]. Accordingly, responders to acute vasodilator testing have to show a decrease of the mean pulmonary arterial pressure of more than 20% from baseline, and the mean pulmonary arterial pressure has to be less than 40mmHg. Only responders defined by these criteria seem to be suitable for long-term treatment with CCB, approximately 12% of the adult iPAH patients. Based on these criteria, at our pediatric heart center patients are treated with CCB when the ratio of the mean pulmonary (PAP) to mean systemic arterial pressure (SAP) decreases by acute vasodilator testing to less than 0.4. Those patients who do not respond initially or acute responders who deteriorate to nonresponders are usually treated with intravenous epoprostenol [6, 7]. However, as the algorithm for treating iPAH is applied, novel agents are used, mostly on a compassionate basis [8].

Considering the pathogenesis of iPAH as a complex and multifactorial process in which endothelial dysfunction as well as severe structural changes of the pulmonary vasculature play an integral role, the aim of the present study was to test the endothelium-dependent pulmonary arterial relaxation (EdPAR) in affected children. Our studies were particularly influenced by the following observations and questions: Why can a small group of patients be treated with CCB long-term, thus utilizing solely pulmonary vasodilator strategy? Why do children respond to short-term vasodilators more often than adults and why is the response of the pulmonary vasculature more likely the younger the child is?

In this context, the important, still unresolved question is whether significant vascular reactivity and better long-term outcome reflect a different stage, a slower degree of disease progression or even a different form of iPAH.

METHODS

In 17 children (Table 1) with iPAH undergoing routine diagnostic cardiac catheterization we studied the endothelium-dependent pulmonary arterial relaxation by acetylcholine, an endothelium-dependent vasodilator. Approval by the institutional review board and written informed parental consent were obtained before including the children in the study.

The following protocol was used:

1) baseline hemodynamic measurements; 2) administration of acetylcholine in graded infusions with a 1000-fold dose range to determine the maximal response; 3) washout time for 15 minutes; 4) administration of 4-6 l oxygen continuously per nasal prongs and epoprostenol infusions or iloprost inhalation, respectively.

After baseline hemodynamic assessment a 7F guide-catheter (Cordis) was placed into the left lower lobe vessel. A Doppler-tip 0.014-in Flow-wire (Cardiometrics) was positioned through an infusion or guide catheter, just distal to the tip of the catheter. Serial 3-minute infusions of acetylcholine (flow rate 0,8ml/min) were administered. Infusions were adjusted to the local blood flow, as calculated from the vessel area and blood flow velocity, in order to achieve 10-7, 10-6, 10-5, and 10-4 M concentrations of acetylcholine in the investigated vessel. The diameter of the pulmonary segmental arteries and the pulmonary blood flow velocity as average peak velocity (APV, cm/s) were measured using quantitative angiography and an intra-arterial Doppler-Flow-wire, as described in detail previously [9, 10].

To avoid any artifacts during hemodynamic assessment by acetylcholine infusion only additional online-registered parameters were considered: APV with continuously calculated pulmonary flow reserve (PFR), heart rate (beats /min), transcutaneous arterial oxyhemoglobin saturation (%) continuously measured by pulse oxymetry (Critikon), PAP (mmHg) and SAP (mmHg). The ratio of mean PAP and SAP (PAP/SAP) was calculated.

Iloprost (Schering) was prepared from a vial of 50µg and diluted with NaCL 0.9% to obtain a 0.5µg/kg solution, which was actively inhaled in awake patients or via mask by a microprocessor controlled Optineb®-inhaler (Nebu-Tec). Short-term infusion with prostacyclin (Epoprostenol, Glaxo-Smith-Kline) was administered intravenously. The infusion started with 5 ng/kg/min and was increased stepwise by 5 ng/kg/min every 5-10min to a maximal dose of 15 to 20 ng/kg/min.

STATISTICAL ANALYSIS

Flow velocities were the primary end points in this study, and were used as an index of resistance-vessel function. The vessel diameter at the site of Doppler velocity sampling was measured to ensure that changes in flow velocity were not attributable to changes in vessel diameter. Similarly, pulmonary arterial and systemic blood pressures were registered to demonstrate that changes in flow velocity were not due to changes in trans-pulmonary pressure.

All data were analyzed using the GraphPad Prism Software package. Results are expressed as mean ± SD. An analysis was performed for all patients and separately for responders and nonresponders. For normally distributed data repeated measures ANOVA and for non-normally distributed data the Friedman's test was used for each parameter, with post hoc corrections as appropriate, for all pairwise multiple comparisons. $P<0.05$ was considered significant. Sub group data containing less than five patients are only described in mean values and minimum / maximum range.

RESULTS

The demographic and hemodynamic patient data are summarized in Table 1.

Depending on the response to short-term oxygen/prostanoid vasodilator testing seven patients aged 0.5 – 18 years showed an acute response to vasodilator testing (Fig. 1a). The mean pulmonary artery pressure fell by 34.2 mmHg from 62.1 ± 21.9 to 27.9 ± 12 mmHg ($p = 0.01$). The mean systemic pressure did not change (p = n.s.). The ratio of PAP/SAP decreased significantly ($p = 0.01$) from 0.9 ± 0.23 to 0.31 ± 0.11.

Ten children aged 0.7 – 12 years did not have a response to acute vasodilator testing (Fig. 1b). The mean PAP/SAP ratio before treatment was calculated to be 1.0 ± 0.2, and remained almost unchanged during the combined vasodilator therapy.

Acetylcholine Assessment: The mean average peak velocity at baseline was 17.6 ± 4.9 cm/s for the entire group. During acetylcholine infusion, the flow velocity increased by 117% to 38.8 ± 28.5 cm/s ($p = 0.01$). In all patients with a global endothelium-independent response to oxygen/prostanoid testing acetylcholine induced an increase of local pulmonary flow from 18.4 ± 4.5 cm/s at baseline to 62.4 ± 35.5 cm/s (Fig. 1c). The average increase of flow velocity was 294%, reflecting a significant rise in PFR to 3.6 ± 2.0 ($p = 0.01$). The change in pulmonary arterial flow velocity in response to four doses of acetylcholine in one patient (No. 2) is shown in Figure 2.

In patients not responding to acute vasodilator testing, a non-uniform behavior of the vasculature was observed (Fig. 1d). The mean baseline APV of 17.0 ± 5.5 cm/s of all nonresponders did not differ significantly from the mean baseline flow velocity of the responder group ($p = 0.1$), and upon acetylcholine stimulation the APV increased to 22.3 ± 10.2cm/s, which was not significant ($p = 0.2$). At a closer look, in five of these 10 patients (Pts 12 to 17) acetylcholine did not have any effect at all, but in the other five patients an increase of the APV from a baseline of 20.2cm/s (range 12 to 23 cm/s) to a maximum of 31cm/s (range 26 to 37 cm/s), corresponding to a PFR of 1.6 ± 1.1, could be demonstrated.

Table 1. Demographic and hemodynamic patient data.

pt.	r	age	weight	sex	NYHA	SaO_2	SvO_2	RAP	PAP	SAP	PAP/SAP	*PAP/SAP*	APV	*APV*	PFR	diameter	outcome
		years	kg			%	%	mmHg	mmHg	mmHg	mmHg	*mmHg*	cm/s	*cm/s*	ratio	mm	
											base	*max*	base	*peak*			
1	x	8,1	37	m	IV	91	69	6	57	74	0,79	*0,24*	18	*114*	6,3	4,3	HLTX
2	x	18	55	f	II	98	70	5	59	91	0,65	*0,32*	13	*80*	6,2	4,8	NYHA II
3	x	4,3	20	m	IV	94	71	4	95	81	1,17	*0,56*	25	*90*	3,6	3,5	NYHA II
4	x	4,4	15	f	III	95	72	3	38	50	0,68	*0,31*	13	*45*	3,5	3,3	NYHA II
5	x	0,7	6	f	IV	92	55	5	90	71	1,2	*0,33*	17	*46*	2,7	3,1	NYHA II
6	x	0,5	6	m	IV	91	64	2	49	52	0,95	*0,27*	21	*33*	1,6	2,9	NYHA II
7	x	13,7	54	f	II	94	74	6	47	79	0,59	*0,38*	22	*29*	1,3	4,2	NYHA II
mean		**7,1**	**27,6**	(3m/4f)		**93,6**	**67,9**	**4,4**	**62,1**	**71,1**	**0,9**	***0,3***	**18,4**	***62,4***	**3,6**	**3,7**	
SD		**6,6**	**21,2**			**2,5**	**6,5**	**1,5**	**21,9**	**15,1**	**0,2**	***0,1***	**4,5**	***32,4***	**2,0**	**0,7**	
8	o	10,1	27	f	III	97	69	4	78	74	1,05	*0,94*	12	*26*	2,2	4,5	died (38m after d.)
9	o	5,1	16	m	II	93	61	7	84	72	1,16	*1,10*	23	*37*	1,6	3	NYHA II
10	o	6,4	16	m	IV	98	67	5	62	88	0,7	*0,76*	22	*34*	1,5	3,1	NYHA II
11	o	7,1	22	f	III	92	68	2	65	78	0,83	*0,88*	21	*28*	1,3	4,1	NYHA III
12	o	9	41,3	m	II	94	70	6	83	85	0,98	*0,69*	23	*30*	1,3	4,1	f/u too short
13	o	2,3	10	f	IV	95	53	6	75	70	1,10	*1,15*	12	*14*	1,2	2,3	died (16mo after d.)
14	o	0,7	9,2	m	IV	96	70	4	93	76	1,22	*1,19*	21	*21*	1,0	3,1	f/u too short
15	o	8,7	15	f	IV	91	53	5	47	54	0,87	*0,79*	15	*15*	1,0	3,5	died (5.3mo after d.)
16	o	2,3	11	m	IV	92	60	6	72	74	0,97	*0,83*	9	*8*	0,9	3,2	died (13mo after d.)
17	o	12	40	f	IV	91	59	7	137	97	1,56	*1,51*	12	*10*	0,8	4,7	died (23mo after d.)
mean		**6,4**	**20,8**	(5m/5f)		**93,9**	**63,0**	**5,2**	**79,6**	**76,8**	**1,0**	***1,0***	**17,0**	***22,3***	**1,3**	**3,6**	
SD		**3,7**	**11,8**			**2,5**	**6,7**	**1,5**	**24,0**	**11,6**	**0,2**	***0,3***	**5,5**	***10,2***	**0,4**	**0,8**	

mean and SD of all patients: x = responder, o = non-responder

mean		**6,7**	**23,6**	(8m/9f)		**93,8**	**65,0**	**4,9**	**72,4**	**74,5**	**1,0**	***0,7***	**17,6**	***38,8***	**2,2**	**3,6**	
SD		**4,8**	**15,6**			**2,4**	**6,7**	**1,5**	**23,4**	**12,6**	**0,2**	***0,4***	**4,9**	***28,5***	**1,7**	**0,7**	

APV = average peak velocity (cm/s), max = maximum
d. (in 'outcome') = diagnosis (not: first symptoms)
diameter = diameter of vessel, in which flow-wire was placed
f/u = follow up
HLTX = heart-lung-transplantation
m = male, f = female
max = PAP/SAP after maximal vasodilatation
mo = month
NYHA = New York Heart Association (classification)
PAH = pulmonary arterial hypertension
PAP = mean pulmonary arterial pressure; SAP = mean systemic arterial pressure
peak = peak average flow velocity
r = responder status (x = responder/o = non-responder)
RAP = right atrial pressure
SaO_2 = arterial oxygen satuaration; SvO_2 = mixed venous oxygen saturation
SD +/- = standard deviation

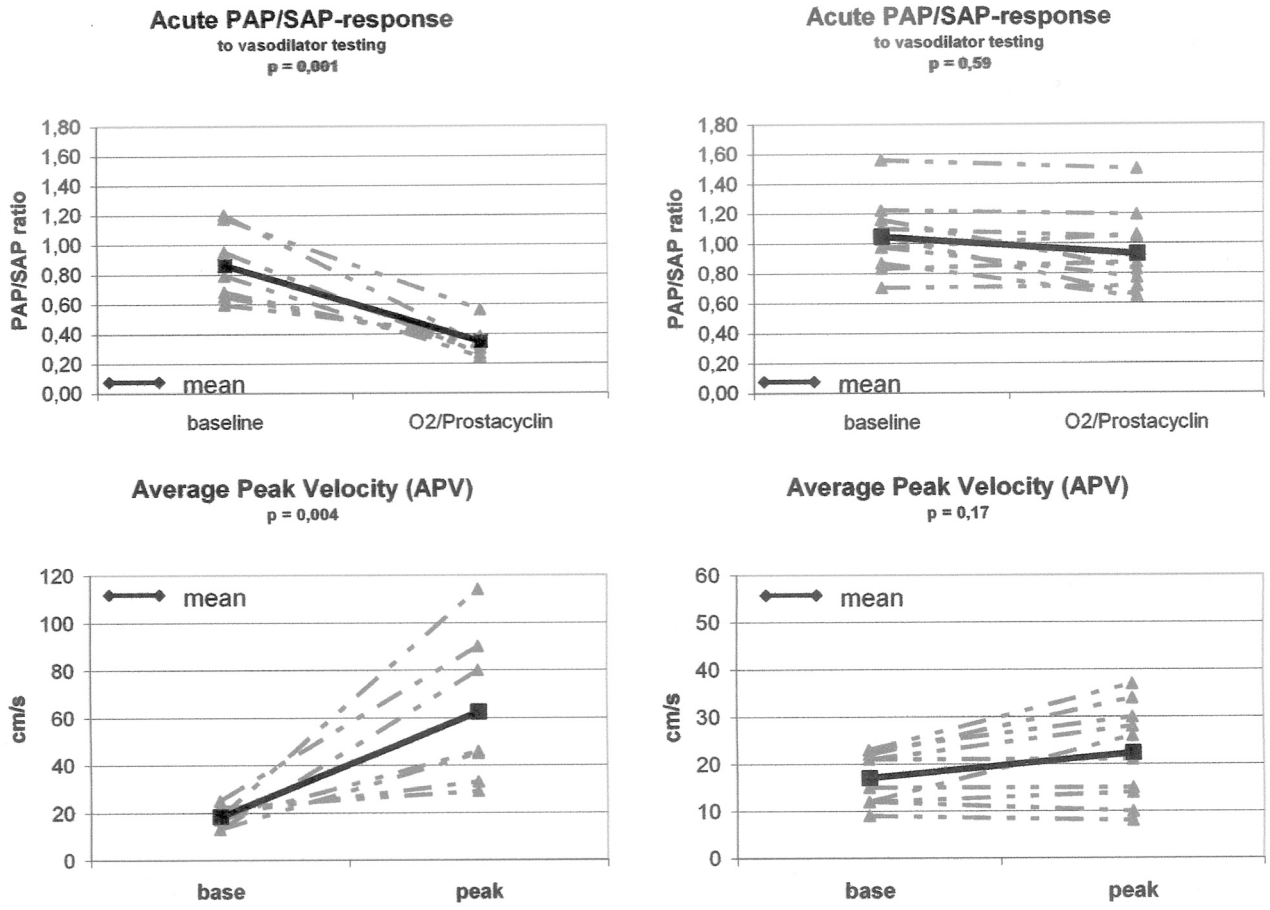

Fig. 1. Plot of change in the ratio of mean pulmonary artery pressure (PAP) and mean systemic arterial pressure (SAP) as an acute response to combined oxygen / prostanoid testing, and pulmonary arterial flow velocities in response to acetylcholine with maximal local concentration (10-4M). Figure 1a represents the responders to acute testing (n = 7); depicted is the impressive decrease of the PAP/SAP-ratio (mean values). Figure 1b shows the data of the non-responder group (n = 10), and figure 1c the marked increase of pulmonary arterial flow velocity in response to the highest dose of acetylcholine in the responder-group (responder to oxygen/prostanoid). In figure 1d the mean APV and the flow velocities in the pulmonary arteries of each patient of the non-responder-group are presented.

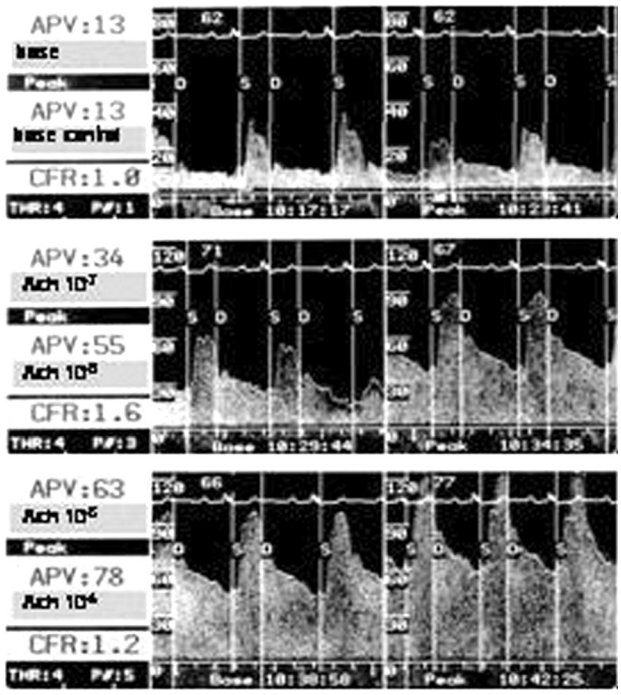

Fig. 2. Shown is the average peak velocity (APV) at baseline and in response to four doses of acetylcholine (estimated local concentrations of 10-7, 10-6, 10-5 and 10-4 M, respectively) in patient 2 who was also a responder during the oxygen/prostanoid test. APV increased from 13 to 34 to 55 to 63 to 80 cm/s. CFR: coronary (i.e. pulmonary) flow reserve.

Prior to the acetylcholine infusion the internal diameter of the segmental pulmonary vessel averaged 3.6 ± 0.74mm in all patients and remained unchanged 3.5+/-0.83mm during the highest concentration of acetylcholine. At the same time no hemodynamic effects were observed during the acetylcholine infusion in respect to heart rate, PAP and SAP values; the transcutaneous arterial oxyhemoglobin saturation remained unchanged as well.

Four children of the non-responder group in whom the APV did not show any change during acetylcholine infusion died despite long-term epoprostenol and, in some patients, additional bosentan therapy during a follow-up period of 5.3 – 23 (mean of 14.5 months, Table 1).

In contrast, the other five non-responders in whom the APV increased during acetylcholine infusion remained stable or improved clinically, although one died by a catheter related sepsis (Pt 8). Interestingly, three further children improved clinically after additional treatment with sildenafil, and two (Pts. 9 and 10) regained vascular reactivity documented by follow-up catheterizations after 20 and 34 months, respectively.

Discussion

Our study demonstrates that endothelium-dependent pulmonary arterial relaxation in children with iPAH is not only preserved in those with an acute response to global endothelium independent vasodilator testing, but even shows an excess compared to previously published data in children and adults with normal hemodynamics [9, 10]. The novel diagnostic tool of in vivo testing of endothelial function by determination of the pulmonary flow reserve casts a light on the pathogenesis and has implications for therapy at our institution. The method of quantitative vascular angiography combined with Doppler flow measurements in response to graded concentrations of acetylcholine or other agonists is the "gold standard" when it comes to assessing endothelial function in human arteries [11].

Acetylcholine induces endothelium-dependent relaxation by receptor-mediated stimulation of endogenous NO [12], PGI2 [13], and perhaps other endothelial cell-dependent vasodilators. Most previous studies have been performed to elucidate the functional status of the coronary endothelium [11], and only few data are available in the setting of iPAH. The endothelium-dependent pulmonary arterial reactivity in children with iPAH has not been examined. In this context the present study demonstrates that EdPAR in children with iPAH is not only preserved in most children with an acute vasodilatory response by oxygen/prostanoid testing, but EdPAR is greater when compared to previously published data in children and adults with normal pulmonary hemodynamics [9, 10]. In healthy children Celermajer et al. [9] showed a maximal increase of the flow velocity in response to acetylcholine of 93 ± 7%. In children with pulmonary vascular disease as a result of congenital heart disease [14] the flow velocity response to acetylcholine was impaired. However, the utilized technique did not allow to determine the absolute values of basal flow velocity in segmental pulmonary arteries of children [9, 14, 15]. Cooper et al. [10], evaluating APV in seven healthy adults using the same method and acetylcholine concentrations as those in our study, demonstrated an increase of the local pulmonary flow by 119% from a baseline level of 6 cm/s to a mean to 14 cm/s. Comparing these data, most children with iPAH in our study had a distinctly higher baseline of the pulmonary arterial flow velocity (mean APV of 17.6 +/-4.9 cm/s). Because locally administered acetylcholine did not induce any change in the segmental artery diameter, as it was also shown in previous studies [10, 15, 16], the site of endothelium-dependent relaxation must be distal to the level of the conduit vessels. In addition, the increase in flow velocity could not be attributed to a rise in pulmonary artery pressure since heart rate, arterial oxygen saturation, systemic arterial and pulmonary arterial pressures remained unchanged.

In respect to the acute vasodilator testing using oxygen combined with an inhaled or intravenously applied prostanoid the non-responders showed a non-uniform response to acetylcholine. Five patients did not respond at all; the other five children had a preserved EdPAR. In this context it has to be considered that endothelial dysfunction can manifest itself either by decreased secretion of vasodilators, increased production of vasoconstrictors, and increased sensitivity to vasoconstrictors and/or resistance of smooth muscle cells to endothelial vasodilators. Vascular remodeling might be the consequence, but can also cause endothelial dysfunction [17]. Therefore the identification of patients with an acute response to the oxygen/prostanoid testing accompanied with an excessive endothelium-mediated flow reserve on the one hand and of patients with preserved EdPAR and those deficient in this endothelial function on the other hand raises the question, whether we deal with different forms of pulmonary vascular diseases.

The excessive response to acetylcholine in some patients with iPAH demonstrates the principal availability of endogenous NO perhaps due to increased expression of endothelial NO synthase (eNOS), but refutes some reports in the literature that endothelial mediated vasodilatation is invariably reduced in patients with iPAH [3, 18]. It would be interesting to know whether these are the patients without any endothelial response to acetylcholine as well as without any acute vasodilator response to oxygen-prostanoid administration. The differences in the outcome of our functional test supports other observations reporting in human lungs strong immunostaining of eNOS in the endothelium of abnormally thickened medium and small pulmonary arteries that otherwise showed typical characteristics of pulmonary hypertension [18]. This important paradox in the literature that eNOS expression has been reported to be decreased, unchanged, or increased [19] might be clarified only by a prospective study comparing functional tests with representative tissue samples removed from human lungs for immunostaining of eNOS.

In conclusion, our data support our hypothesis of different subtypes of iPAH. The excessive response to acetylcholine in several patients not only demonstrates the principle availability of endogenous nitric oxide, but also suggests that the excessive EdPAR might be

useful to choose those patients who are suitable for long-term treatment with CCB. In addition, we speculate that patients with a small, but detectable, EdPAR may predominantly have a disease of endothelial dysfunction. Untreated vasoconstriction, due to impaired vasodilatation, likely increases shear stress and leads to pulmonary vascular remodeling. This might explain why pulmonary vasodilator treatment alone is successful in treating iPAH in 12% of adults only and about 20% to 40% of children and infants, respectively. Additionally, we believe that patients with impaired, but residual EdPAR might benefit from stimulation of endogenous endothelial vasodilator production rather than from treatment with exogenous substances, which can lead to further down-regulation of endogenous vasoprotective mediators, as it has indeed been shown for NO [20]. Therefore, at least for these patients phosphodiesterase-inhibitors may be more beneficial for long term therapy than agonists like inhaled NO.

The non-responders to acute vasodilator testing likely have a form of pulmonary arterial hypertension as a result of a remodelling of the vessels independent of the endothelium-dependent vasodilator reserve of the resistance vessels. Patients without any endothelial reactivity at all may have a disease which is characterized by abnormal proliferation of pulmonary endothelial and smooth muscle cells, and abnormal adventitial connective tissue. These pulmonary vessels seem to be encased in a rigid matrix of adventitial connective tissue, which may impair vasodilation. For this majority of iPAH patients new anti-angiogenic and antiproliferative strategies need to be developed, since the current drugs to treat this form of iPAH fail in the long term.

Clearly, the implications of our study are limited by the small number of patients; yet this in vivo endothelial function test expands the diagnostic tools in patients with iPAH and may have an impact on therapeutic strategies and prognosis.

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