

Circulating endothelin in children with congenital heart disease

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Abstract

Objective—To evaluate whether circulating endothelin, a peptide that is thought to play a part in mediating vascular tone, might be high in pulmonary hypertensive congenital heart disease.

Design—A prospective study with a radioimmunoassay technique to estimate urinary and plasma endothelin concentrations.

Setting—A supraregional referral centre for patients with congenital heart disease.

Patients—The 12 hour urinary endothelin concentration in young children with an increased pulmonary blood flow ($n = 24$, median age eight months) were compared with those in children with right ventricular outflow tract obstruction ($n = 14$, median age 1.5 years) and with those in healthy controls ($n = 16$, median age 1.8 years). The concentrations were also measured in adolescents with irreversible pulmonary vascular disease ($n = 17$, median age 18 years) and compared with those in controls of similar age ($n = 19$, median age 18.5 years). Also the plasma concentrations in the left atrium and pulmonary artery were measured in young children with either high ($n = 11$, median age 10.8 months) or low ($n = 5$, median age 1.0 year) pulmonary blood flow, in the peripheral arterial and venous blood of young children with either high ($n = 13$, median age 10.8 months) or low ($n = 6$ median age 1.9 years) pulmonary blood flow, and in the peripheral venous blood of seven healthy young children (median age 1.7 years).

Results—The urinary excretion of endothelin was similar in young children of a similar age, whether they had high, low, or normal pulmonary blood flow. Also, urinary endothelin excretion in older patients with irreversible pulmonary vascular disease was similar to that in normal subjects of similar age. Urinary endothelin excretion in normal young children, however, was significantly greater than that in normal older subjects ($p = 0.0001$). There was no transpulmonary or arteriovenous difference detected in either children with high or low pulmonary blood flow, and plasma concentrations sampled from the left atrium, pulmonary artery, and systemic artery and vein were similar in both groups.

Conclusion—There was no evidence to implicate circulating endothelin in the pathogenesis of pulmonary vascular disease.

(Br Heart J 1993;69:233-236)

Endothelin is a long acting vasoconstrictor peptide that is thought to mediate vascular tone, and which may play a part in the pathophysiology of vascular disease associated with vasoconstriction.¹ Cultured pulmonary arterial endothelial cells are capable of producing endothelin and its release is increased in response to shear stress.^{2,3} Endothelin interacts with other vasoactive substances, stimulating the release of endothelin derived relaxing factor, prostacyclin, and thromboxane A₂ in the lung.⁴ Its vasoconstrictor effect on vascular smooth muscle can be attenuated by cyclooxygenase inhibitors and thromboxane receptor antagonists. The vasoconstrictor effect of endothelin is most pronounced when the endothelium is absent.⁵

In pulmonary hypertensive congenital heart disease shear stress, endothelial damage, metabolic dysfunction, and abnormal eicosanoid biosynthesis have all been implicated as contributing factors in the pathogenesis of pulmonary vascular disease.⁶⁻⁸ In our study we investigated the possibility that endothelin concentrations were increased in children with pulmonary vascular disease, by studying young children with a high pulmonary blood flow and older children with irreversible pulmonary vascular disease.

Patients and methods

We measured the 12 hour urinary excretion of endothelin (ET-1,2 and big endothelin (ET-1,2/ET)) and big endothelin (ET-1,2/ET) in young children with congenital heart disease who had either a high pulmonary blood flow or a low flow due to right ventricular outflow tract obstruction (table 1). All but one of these children was less than three years of age. Urinary endothelin concentrations were measured in age matched controls. The 24 hour urinary excretion of endothelin was also measured in a group of older patients with irreversible pulmonary vascular disease and in controls of a similar age. The plasma concentration of ET-1,2/ET was determined in pulmonary arterial and left atrial blood samples drawn at routine cardiac catheterisation in young children with either a

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Accepted for publication
9 September 1992

Endothelin concentrations in different body fluids

Group	1	2	3	4	5	6	7	8	9	10
No	24	14	16	17	19	11	5	13	6	7
Median age	8 months	1.5 yr	1.8 yr	18 yr	18.5 yr	10.8 months	1.0 yr	10.8 months	1.9 yr	1.7 yr
M:F	2:1	1.3:1	1:1	1.4:1	1.4:1	1.2:1	1.5:1	1.2:1	2:1	1.3:1
Pulmonary blood flow	High	Low	Control	1PVD:PVR*Control	High	High	Low	High	Low	Control
Qp:Qs	> 2:1	0.2-0.9 (median 0.6)		5-33 (median 17)		> 2:1	0.5-0.8 (median 0.6)	> 2:1	0.5-0.8 (median 0.65)	
Endothelin concentrations:										
Urinary ET-1,2/ET (mean (SEM)/mg/g creatinine)	318 (25)	332 (50)	391 (60)	113 (9)	106 (8)	—	—	—	—	—
Plasma ET-1,2/ET (mean (SEM) fmol/ml)										
In pulmonary artery	—	—	—	—	—	32 (3.8)	51 (12.6)	—	—	—
In left atrium	—	—	—	—	—	35 (3.7)	48 (3.7)	—	—	—
Peripheral plasma ET-1,2 (mean (SEM) fmol/ml)										
Venous	—	—	—	—	—	—	—	13.9 (0.84)	13.1 (1.3)	14.0 (3.5)
Arterial	—	—	—	—	—	—	—	15.04 (1.5)	14.7 (3.6)	—

*IPVD, irreversible pulmonary vascular disease; PVR, pulmonary vascular resistance (units m^2); Qp, pulmonary blood flow index; Qs, systemic blood flow index.

high or low pulmonary blood flow (without aged matched controls). The assay specific for ET-1,2 then became available. Therefore the concentration of ET-1,2 was determined at cardiac catheterisation in the peripheral arterial and venous blood of young children who had either an abnormally high or low pulmonary blood flow, and in the venous blood of six aged matched controls sampled at a time of intercurrent illness. Also, one child with mild pulmonary valve stenosis (gradient 35 mm Hg) who underwent cardiac catheterisation was included in the control group. None of the patients or controls had systemic hypertension. All patients and control subjects or their parents gave informed consent.

ENDOTHELIN ASSAYS

Urine samples were stored at -20°C until analysis. Blood samples were collected in lithium/heparin tubes, centrifuged at 4°C , and the plasma stored at -20°C until analysis.

Radioimmunoassay of ET-1,2/ET

For both urine and blood samples, ET-1,2/ET was measured with the commercially available Amersham International ET-1,2 ^{125}I assay system. The antibody exhibits a cross reactivity of 100% with ET-1, 37.9% with big ET, 204% with ET-2, and 0.0024% with ET-3 (Amersham International). The urinary concentration of endothelin is expressed in relation to the excretion of creatinine. In all urine samples the creatinine was measured by standard laboratory techniques.

Plasma concentrations of specific ET-1,2

Specific ET-1,2 in plasma was measured with the Amersham International ET1-21 specific ^{125}I assay system. The crossreactivities were: 100% with ET-1, 144% with ET-2, 52% with ET-3, 0.4% with big ET-1, <0.003% with big ET 22-38 (Amersham International).

Final concentrations of endothelin were expressed as ng endothelin/g of creatinine for urine and as fmol/ml for plasma. Results were compared by a Mann-Whitney U test for

unpaired samples and the Wilcoxon signed rank test for paired samples and were considered significant when $2p < 0.05$.

Results

URINARY ENDOTHELIN CONCENTRATION WITH THE ET-1,2/ET ASSAY

The table shows that the mean excretion rate of urinary endothelin was similar in young children with a high and a low pulmonary blood flow ($2p = 0.64$) and in both groups values were similar to those in normal children of similar age ($2p = 0.54$, 0.34 respectively; fig 1). The mean endothelin concentration was lower in older healthy controls than in the young controls ($2p = 0.0001$). In older patients with irreversible pulmonary vascular disease the mean concentration of endothelin excreted was similar to that in aged matched controls ($2p = 0.7$).

PLASMA ENDOTHELIN CONCENTRATION WITH THE ET-1,2/ET ASSAY

There was no significant difference between the mean pulmonary arterial ET-1,2/ET concentration in children with a high pulmonary blood flow and in those with a low pulmonary blood flow ($2p = 0.14$; fig 2). Neither was there a difference in the left atrial samples in these two groups ($2p = 0.11$). There was no difference between pulmonary arterial and

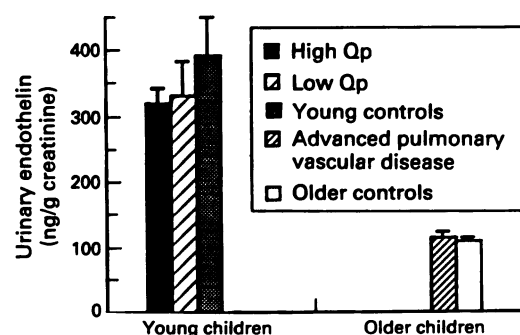


Figure 1 Urinary endothelin concentrations in young and older children with congenital heart disease, each group with its own controls of similar age. Qp, pulmonary blood flow index.

Figure 2 Plasma endothelin concentrations in the pulmonary artery and left atrium of children with a high and low pulmonary blood flow. Q_p , pulmonary blood flow index.

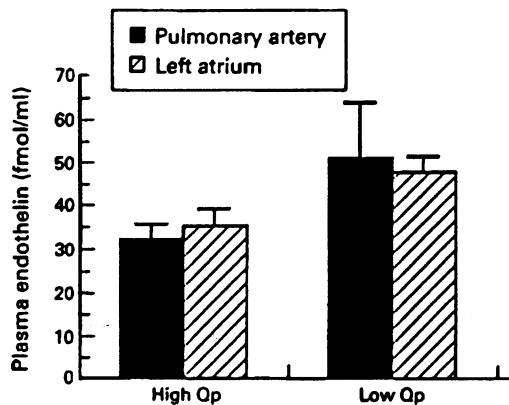
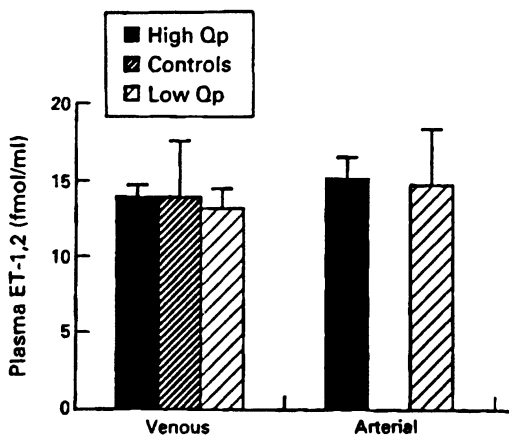


Figure 3 Plasma specific endothelin concentrations in peripheral blood of children with a high and low pulmonary blood flow. Note: arterial samples from normal children were not available. Q_p , pulmonary blood flow index.



left atrial samples in either the patients with a high pulmonary blood flow ($2p = 0.84$) or with a low pulmonary blood flow ($2p = 0.72$).

PERIPHERAL PLASMA ENDOTHELIN CONCENTRATION WITH THE SPECIFIC ET-1,2 ASSAY

There was no statistical difference between the mean venous endothelin concentrations in children in the high flow, low flow, and control groups ($p = 0.8$; fig 3). The mean arterial ET-1,2 concentration was similar in children with a high and a low pulmonary blood flow ($2p = 0.57$). There was no significant difference between the peripheral arterial and venous concentrations in either the children with high flow ($2p = 0.53$) or the children with low flow ($2p = 0.69$). In the single patient in the control group who underwent cardiac catheterisation the concentrations were, in the femoral vein 12.1 fmol/ml, femoral artery 12.6 fmol/ml, and pulmonary artery and pulmonary vein 14.0 fmol/ml.

Discussion

We surmised that in the absence of renal dysfunction the 12 and 24 hour urinary excretion of endothelin might more accurately reflect the basal release of endothelin without the possible confounding influence of vessel cannulation that is known to cause increases of both endothelin and other vasoactive mediators such as eicosanoids.⁹⁻¹¹ It also allowed us to study normal children non-invasively. We

found no difference in endothelin excretion between children with heart disease and healthy controls but did find a noticeable difference between young and older subjects. This could reflect a change in the renal handling of endothelin, which is thought to be partly dependent on tubular function.¹² This is known to undergo maturational changes.¹³ Increased concentrations of vasoactive mediators may be a feature of early childhood and higher plasma concentrations of endothelin have been reported in children less than three months of age.¹⁴ The venous plasma endothelin concentrations of the young children in our series were greater than those in a small group of healthy adult investigators measured in our laboratory. The plasma concentrations of big endothelin were higher in both children with heart disease and controls suggesting that activation of big endothelin to specific endothelin may occur within the circulation.

The fate of endothelin in the lung is controversial and may be species dependent.¹⁵ In the human lung, one recent study suggested net clearance of endothelin by the lung,¹⁵ whereas another did not.¹⁴ We failed to detect a peripheral arteriovenous difference or a difference across the pulmonary vascular bed in 35 children with either a high or low pulmonary blood flow. This finding does not support the concept that there is a net extraction or secretion of endothelin across the human pulmonary vascular bed.

In our study the urinary excretion of endothelin was normal for age in pulmonary hypertensive children, both in young patients with potentially reversible pulmonary vascular changes and in the older patients with irreversible disease. An increase in the systemic arterial and venous concentrations of endothelin has been reported in a group of adults with primary and secondary pulmonary hypertension due to hypoxic lung disease, pulmonary thromboembolism, and the Eisenmenger syndrome.¹⁶ We did not measure plasma endothelin concentrations in our older patients but the normal 24 hour urinary excretion of endothelin does not suggest a consistently high circulating concentration of endothelin. In our young patients the plasma endothelin concentrations were within normal limits. Yoshiyayashi *et al* have reported increased concentrations of plasma endothelin in children with pulmonary hypertension due to congenital heart defects.¹⁴ Their group of 18 patients, however, included five postoperative cases, and we have preliminary data indicating an increased concentration of endothelin after intracardiac repair in young children who had a normal plasma concentration before operation.¹⁷

Although *in vitro* studies indicate that endothelin increases in response to shear stress,^{2,3} we could find no evidence that this is so *in vivo*. This, however, does not necessarily obviate a role for endothelin in the pathogenesis of pulmonary vascular disease. Children with pulmonary hypertension have morphologically abnormal endothelial cells^{6,7} and a resulting change in endothelial cell

smooth muscle interaction might result in increased sensitivity of hypertrophied smooth muscle cells to normal concentrations of the vasoconstricting peptide endothelin.

This study was supported by a grant from the British Heart Foundation

- 1 Yanagisawa MH, Kurihara S, Kimura S, Tombe Y, Kobayashi M, Mitsui Y, *et al.* A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988; 332:411-5.
- 2 Ryan US, Glassberg MK, Noland KB. Endothelin-1 from pulmonary artery and microvessels acts on vascular and airway smooth muscle. *J Cardiovasc Pharmacol* 1989; 13:S57-62.
- 3 Yoshizumi M, Kurihara H, Sugiyama T, Takaku F, Yanagisawa M, Masaki T, Yazaki Y. Hemodynamic shear stress stimulates endothelin production by cultured endothelial cells. *Biochem Biophys Res Commun* 1989; 161:859-64.
- 4 De Nucci G, Thomas T, D'Orleans-Juste P, Antunes E, Walder C, Warner TD, Vane JR. Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelin derived relaxing factor. *Proc Nat Acad Sci USA* 1988; 85:9797-800.
- 5 Hiley CR, Douglas SA, Randall MD. Pressor effects of endothelin-1 and some analogs in the perfused superior mesenteric arterial bed of the rat. *J Cardiovasc Pharmacol* 1989; 13:S197-9.
- 6 Rabinovitch M, Bothwell T, Hayakawa BN, Williams WG, Trusler GA, Rowe RD, *et al.* Pulmonary artery endothelial abnormalities in patients with congenital heart defects and pulmonary hypertension. A correlation of light with scanning electron microscopy and transmission electron microscopy. *Lab Invest* 1986;55: 632-53.
- 7 Hall SM, Haworth SG. Onset and evolution of pulmonary vascular disease in young children : abnormal postnatal remodelling studied in lung biopsies. *J Pathol* 1992; 166:183-94.
- 8 Adatia IT, Haworth SG. Advanced pulmonary vascular disease: 24 hour urinary excretion of vasoactive eicosanoids. *Circulation* 1990; 82 (suppl III):A0395.
- 9 Hirata Y, Itoh K, Ando K, Endo M, Marumo F. Plasma endothelin levels during surgery. *N Engl J Med* 1989; 321:1686.
- 10 Roy L, Knapp H, Robertson R, FitzGerald G. Endogenous biosynthesis of prostacyclin during cardiac catheterisation and angiography in man. *Circulation* 1985; 71:434-40.
- 11 Nowak J, FitzGerald G. Redirection of prostaglandin endoperoxide metabolism at the platelet-vascular interface in man. *J Clin Invest* 1989; 83:380-5.
- 12 Ohta K, Kirata Y, Shichiri M, Kanno K, Emori T, Tomita K, Marumo F. Urinary excretion of endothelin-1 in normal subjects and patients with renal disease. *Kidney International* 1991; 39:307-11.
- 13 McCrory WW. Renal structure and development. In: Holliday M A, Barratt T M, Verrier R L, eds. *Pediatric Nephrology*. Baltimore: Williams and Wilkins, 1987; 31-44.
- 14 Yoshiyoshi M, Nishioka K, Nakao K, Saito Y, Matsumura M, Ueda T, *et al.* Plasma endothelin concentrations in patients with pulmonary hypertension associated with congenital heart defects. *Circulation* 1991; 161:2280-5.
- 15 Pernow J, Hemsén A, Lundberg JM. Tissue specific distribution, clearance and vascular effects of endothelin in pig. *Biochem Biophys Res Commun* 1989; 161:647-53.
- 16 Stewart DJ, Levy RD, Cernacek P, Langleben D. Increased plasma endothelin-1 in pulmonary hypertension: marker or mediator of disease? *Ann Intern Med* 1991; 114:464-9.
- 17 Komai H, Adatia IT, Elliott MJ, DeLeval MR, Haworth SG. Increased endothelin plasma levels following cardiopulmonary bypass in congenital heart disease. *Am Rev Respir Dis* 1992; A641.