PAEDIATRIC CARDIOLOGY

Abnormalities in the biosynthesis of thromboxane A_2 and prostacyclin in children with cyanotic congenital heart disease

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Abstract

***Background*—Children with cyanotic congenital heart disease and pulmonary outflow tract obstruction have shortened platelet survival times and are susceptible to thrombosis and organ infarction. Thromboxane A_2 and prostacyclin have opposing actions on platelet aggregability and an imbalance in their biosynthesis might contribute to the pathophysiology of these complications.**

***Methods*—Biosynthesis of thromboxane A_2 and prostacyclin was investigated in 16 children (4–32 months, median 18 months) with cyanotic congenital heart disease and pulmonary outflow tract obstruction and compared with 16 healthy children of a similar age (6–34 months, median 24 months). Urinary excretion of 2,3-dinor-thromboxane B_2 (a metabolite of thromboxane A_2) and of 2,3-dinor-6-oxo-prostaglandin $F_{1\alpha}$ (a metabolite of prostacyclin) was measured.**

***Results*—The children with cyanotic congenital heart disease and pulmonary outflow tract obstruction excreted more 2,3-dinor-thromboxane B_2 than the healthy children: 916(163) compared with 592(122) ng/g creatinine (mean(SEM); 2p = 0·014). The ratio of excretion of 2, 3-dinor-thromboxane B_2 to 2, 3-dinor-prostaglandin $F_{1\alpha}$ was greater in the patients than in the healthy control group (2·38(0·28) *v* 1·3(0·22)) (2p = 0·002).**

***Conclusion*—The balance between biosynthesis of prostacyclin and of thromboxane A_2 is abnormal in children with cyanotic congenital heart disease and pulmonary outflow tract obstruction and favours platelet aggregation and vasoconstriction.**

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Children with cyanotic congenital heart disease and a low pulmonary blood flow owing to right ventricular outflow tract obstruction may have structurally abnormal hypoplastic pulmonary vessels,[1 2] decreased platelet survival times,[3-5] and rheological abnormalities rendering them susceptible to thrombosis and organ infarction.[6-8] Thromboxane A_2 (a vasoconstrictor and promoter of platelet aggregation) and prostacyclin (a vasodilator and inhibitor of platelet aggregation) are derivatives of arachidonic acid metabolism.[9-11] An imbalance between the biosynthesis of thromboxane and the biosynthesis of prostacyclin has been implicated in several vascular disorders[11-13] and might contribute to the pathophysiology of the complications of cyanotic congenital heart disease with an inadequate pulmonary blood flow. The association of a reduced flow and activated platelets could lead to a disordered interaction between the endothelium and platelets.

A non-invasive approach to studying thromboxane and prostacyclin biosynthesis in vivo is to measure excretion of the metabolites 2,3-dinor-thromboxane B_2 and 2,3-dinor-6-oxo-prostaglandin $F_{1\alpha}$.[14 15] These metabolites reflect extrarenal systemic biosynthesis of thromboxane A_2 and prostacyclin respectively.[16 17] This method avoids the problem of artefactual stimulation of eicosanoid biosynthesis that can occur as a result of endothelial trauma and platelet activation during blood sampling.[18-20] We therefore studied eicosanoid biosynthesis in children with cyanotic congenital heart disease and pulmonary outflow tract obstruction by measuring the 12 hour urinary excretion of 2,3-dinor-thromboxane B_2 and 2,3-dinor-6-oxo-prostaglandin $F_{1\alpha}$.

Patients and methods

We studied 16 children (aged 4–32 months, median 18 months) with cyanotic congenital heart disease caused by right ventricular outflow tract obstruction (table). All children had a systemic blood pressure of < 110/70 mm Hg measured by cuff sphygmomanometry. Twelve-hour bagged urine specimens were collected during the hospital admission the night before cardiac catheterisation or operation. A control group consisting of 16 healthy normotensive control children (nine male and seven female aged 6–34 months, median 24 months) was recruited from the Great Ormond Street Hospital staff crèche and 12-hour overnight bagged urine samples were collected similarly. Parents of all patients and controls gave informed consent. No patient or control subject took aspirin or other non-steroidal anti inflammatory drug in the two weeks before the study and all urine samples were found to be free of salicyluric acid.[21] Concentrations of 2,3-dinor-6-oxo-prostaglandin $F_{1\alpha}$ and 2,3-dinor-thromboxane B_2 in the urine were measured by immunoaffinity chromatography and gas chromatography mass spectrometry as described elsewhere.[22] Briefly, a well mixed

Clinical observations

Case no	Sex	Age (mth)	Diagnosis	Qp:Qs	AO Sat (%)	Platelets ($\times 10^3/mm^3$)	Hb (g/dl)	Drugs	BP (mm Hg)
1	M	4	PAT, VSD	NA	75	259	17·6	Nil	90/50
2	M	5	TOF	NA	81	696	14·7	Propranolol	80/45
3	M	7	TOF	0·9	86	501	12·7	Propranolol	80/50
4	M	8	VSD,PA band	0·7	84	563	12·3	Nil	95/60
5	F	11	TOF	0·7	70	367	14·4	Propranolol	102/60
6	M	11	TOF	0·4	71	170	19·3	Nil	100/65
7	M	11	TGA, VSD, PS	NA	78	223	12·7	Nil	95/50
8	M	18	TOF	0·4	75	360	16·6	Nil	105/65
9	M	18	TOF	0·8	85	264	14·4	Nil	104/75
10	F	21	PAT, IVS, RBT	0·6	84	236	13·4	Nil	95/50
11	F	22	PAT, VSD, RBT	0·6	75	144	19·3	Nil	100/75
12	F	24	TA, PS, VSD, LBT	0·5	74	235	16	Nil	102/50
13	F	25	TOF, RBT	0·5	76	311	14·3	Nil	104/47
14	F	26	TOF, RBT	NA	65	253	14·8	Nil	85/50
15	M	28	TGA, VSD, PS, RBT	0·7	70	250	19·6	Nil	110/60
16	M	32	TOF, R&LBT	0·2	45	48	17·5	Nil	100/60
Median	-	18	-	0·6	75	256	14·8	-	100/55

TOF, Tetralogy of Fallot; VSD, ventricular septal defect; IVS, intact ventricular septum; R/LBTS, right/left modified Blalock-Taussig shunt; PA, pulmonary artery; PAT, pulmonary atresia; PS, pulmonary stenosis; TGA, transposition of the great arteries; AO Sat, oxygen saturation; Hb, haemoglobin; Qp:Qs, pulmonary to systemic flow ratio; NA, not available.

sample of 30–50 ml urine was stored at −20°C until assay. Urine samples (10 ml) were diluted 1:1 by volume with buffer at pH 8·0 and [$^2H_4$] 2,3-dinor-thromboxane B_2 and [$^2H_4$] 2,3-dinor-6-oxo-prostaglandin $F_{1\alpha}$ (5 ng each) were added. Eicosanoids were extracted on cyanogen bromide-activated sepharose columns containing immobilised antibodies that had been raised against 6-oxo-prostaglandin $F_{1\alpha}$ and thromboxane B_2 and that cross-reacted with their respective 2,3-dinor metabolites. Urine samples were applied under vacuum to the columns, which were washed with water (10 ml). Eicosanoids were eluted by addition of 2 × 0·5 ml acetone:water (95:5) and rotation of the columns for 15 min. Samples were taken to dryness (N_2 stream) and were derivatised as 3,5-bis-trifluoromethylbenzyl esters and trimethylsilyl ethers.[23] They were analysed by a VG 70-SEQ gas chromatograph/mass spectrometer in the electron capture mode with methane or ammonia as the reagent gas. Carboxylate anions at mass/charge (m/z) ratio of 557 were monitored for 2,3-dinor-6-oxo-prostaglandin $F_{1\alpha}$ and 2,3-dinor-thromboxane B_2 and at m/z 561 for the deuterated internal standards. The detection limit for each eicosanoid was 5 pg/ml. Urinary creatinine concentrations were measured by standard methods.

Results are expressed as mean (SEM) ng eicosanoid per g creatinine. The Mann-Whitney U test was used in statistical analysis and differences were regarded as significant when $2p < 0·05$.

Results

The patients with cyanotic congenital heart disease excreted significantly more 2,3-dinor-thromboxane B_2 than the control group: 916(163) compared with 592(122) ng/g creatinine ($2p = 0·014$) (fig 1). Excretion of 2,3-dinor-6-oxo-prostaglandin $F_{1\alpha}$ was 381 (61) in the cyanotic children compared with 589 (95) ng/g creatinine in the controls ($2p = 0·08$) (fig 2). The ratio of 2,3-dinor-thromboxane B_2 to 2,3-dinor-6-oxo-prostaglandin $F_{1\alpha}$ was significantly greater in the patients with heart disease (2·38 (0·28)) than in the healthy children (1·3 (0·22)) ($2p = 0·002$) (fig 3). Among the cyanotic children there was no correlation between excretion of 2,3-dinor-thromboxane B_2 and 2,3-dinor-6-oxo-prostaglandin $F_{1\alpha}$ or their ratio and the platelet count, haemoglobin, or pulmonary to systemic flow ratio (Qp:Qs).

Discussion

We showed an increase in the urinary excretion of 2,3-dinor-thromboxane B_2 and in the ratio of urinary 2,3-dinor-thromboxane B_2 to

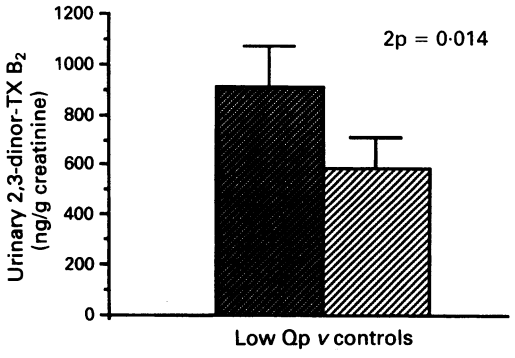

Figure 1 Excretion of 2,3-dinor-thromboxane B_2 (ng/g creatinine) in children with a low pulmonary blood flow (Qp) and in controls. TX thromboxane.

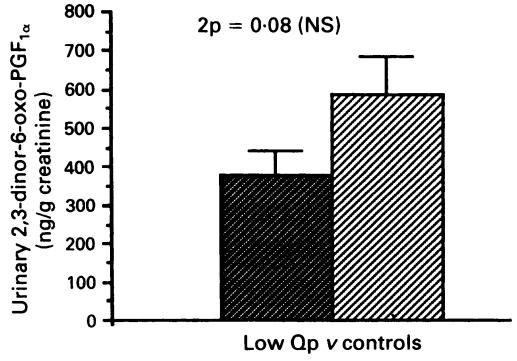

Figure 2 Excretion of 2,3-dinor-6-oxo-prostaglandin $F_{1\alpha}$ (ng/g creatinine) in children with a low pulmonary blood flow (Qp) and in controls. PG, prostaglandin.

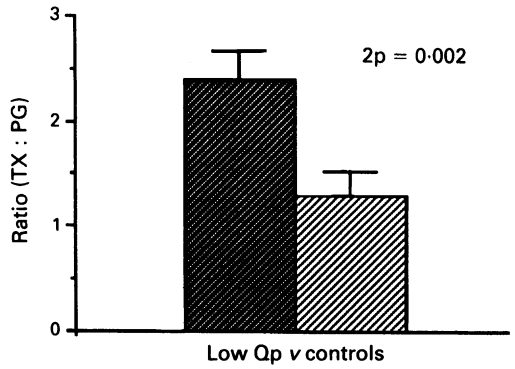

Figure 3 Ratio of 2,3-dinor-thromboxane B_2 to 2,3-dinor-6-oxo-prostaglandin $F_{1\alpha}$ in children with a low pulmonary blood flow (Qp) and in controls. TX, thromboxane; PG, prostaglandin.

2,3-dinor-6-oxo-prostaglandin $F_{1\alpha}$ in young children with cyanotic congenital heart disease and pulmonary outflow tract obstruction. The main source of thromboxane A_2 excreted as 2,3-dinor-thromboxane B_2 is thought to be activated platelets though this eicosanoid may also be produced by endothelial cells and macrophages.[24 25] It seems likely that in cyanotic congenital heart disease, increased excretion of 2,3-dinor-thromboxane B_2 is primarily of platelet origin as there is evidence of abnormal platelet function in such children.[7] Four of our patients had abnormal platelet counts (table 1). In three patients with mild systemic arterial oxygen desaturation the counts were high (> 500 000 per mm^3). In one patient, with the lowest arterial oxygen saturation, the platelet count was low (48 000 per mm^3). Though the patient with the lowest platelet count excreted the most 2,3-dinor-thromboxane B_2, in the group as a whole there was no correlation between 2,3-dinor-thromboxane B_2 and the platelet count. Thrombocytopenia is a late event in cyanotic congenital heart disease. It usually accompanies severe hypoxaemia and polycythaemia.[3] With the contemporary practice of early palliation or corrective surgery thrombocytopenia is now an uncommon finding in young patients. Platelet half times are, however, known to be reduced in patients who have cyanotic congenital heart disease with mild to moderate arterial oxygen desaturation but there is a compensatory increase in platelet production that maintains platelet numbers in the normal or higher than normal range.[3 4]

The increased biosynthesis of thromboxane A_2 in the patients in this study, most of whom had normal platelet counts, could therefore reflect increased platelet activation with a compensatory rise in thrombocyte production. An increase in platelet activation may also explain the paradoxical clinical observation that thrombotic episodes are seen more commonly in children under two years of age before the development of severe polycythaemia and thrombocytopenia.[7] A negative correlation between 2,3-dinor-thromboxane B_2 and platelet count has been reported previously in women with pregnancy-induced hypertension.[26] This is an acute disease, however, in which compensatory mechanisms may not be effective, unlike the slower progression of hypoxaemia caused by worsening right ventricular outflow tract obstruction in congenital heart malformations. Although increased thromboxane biosynthesis is probably due mainly to platelet dysfunction, in vitro studies suggest that the exposure of the endothelium to reduced oxygen tension might also be relevant.[27-31]

We conclude therefore that in children with cyanotic congenital heart disease and a low pulmonary blood flow there is an increase in thromboxane A_2 biosynthesis and in ratio of thromboxane A_2 to prostacyclin. We suggest that these abnormalities precede the development of severe polycythaemia and thrombocytopenia. The increase in the ratio of thromboxane A_2 to prostacyclin which favours vasoconstriction and platelet aggregation may contribute to the development of thrombotic episodes in such children. The findings also support the current practice of early correction, rather than palliation, because even mild to moderate systemic desaturation seems to promote the biosynthesis of thromboxane A_2. The presence of abnormally high thromboxane A_2 production provides a therapeutic rationale for the widely used practice of prescribing aspirin to maintain the patency of systemic to pulmonary artery shunts in those children with unfavourable anatomy who require palliation as an initial procedure.

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