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ORIGINAL PAPER

Plasma endothelin-1 and nitrate levels in Down's syndrome with complete atrioventricular septal defect-associated pulmonary hypertension: a comparison with non-Down's syndrome children

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Abstract Children with Down's syndrome (DS)-associated complete atrioventricular septal defect (AVSD) have rapid and aggressive development of pulmonary vascular disease when compared with non-Down's syndrome (ND) children. We aimed to evaluate the role of plasma endothelin-1 (ET-1) and nitrate levels in DS children with complete AVSD-associated pulmonary hypertension (PH) and compare this to ND patients. The study included 20 patients (11 males, nine females) who had complete AVSD associated with PH. Comparisons were made between DS patients (n=12) aged 4 to 8 months (median 5 months) and ND patients (n=8) aged 4 to 12 months (median 7 months). Blood samples were drawn from the inferior vena cava, pulmonary artery, pulmonary vein, and aorta. The plasma ET-1 concentrations of the two groups were compared to the peripheral venous and arterial ET-1 levels, and pulmonary vein nitrate was compared to the peripheral arterial nitrate levels of ten healthy infants. The mean pulmonary artery (PA) pressure and pulmonary vascular resistance (Rp) were significantly higher in the DS group than ND patients, and the pulmonary blood flow (Qp) in ND patients was higher than DS patients. There were no differences between the two study groups in regard to plasma ET-1 and nitrate levels obtained from matched sampling sites. The plasma ET-1 and nitrate levels were significantly higher in both study groups compared to the control subjects. The plasma ET-1 and nitrate levels in DS patients with PH were not different when compared to those of ND patients.

Keywords Down's syndrome · Endothelin-1 · Nitrate · Complete atrioventricular septal defect · Pulmonary hypertension

Abbreviations

AVSD	Atrioventricular septal defect
DS	Down's syndrome
ET-1	Endothelin-1
ND	Non-Down's syndrome
NO	Nitric oxide
PA	Pulmonary artery
PDA	Patent ductus arteriosus
PH	Pulmonary hypertension
Qp	Pulmonary blood flow
Qs	Systemic blood flow
Rp	Pulmonary vascular resistance
Rs	Systemic vascular resistance

Introduction

Children with Down's syndrome (DS) have a high incidence of congenital heart disease and constitute the majority of patients with complete atrioventricular septal defect (AVSD) [13]. It is known that children with DS may develop progressive pulmonary changes earlier than non-Down's syndrome (ND) children who have similar congenital heart defects [4, 14]. It is not well known whether there are intrinsic molecular factors in pulmonary endothelium for accelerated pulmonary vascular

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disease in patients with DS or not. Pulmonary circulation is regulated by a complex network of neural and humoral factors. Endothelin-1 (ET-1) is a 21-amino acid vasoactive polypeptide that is derived from pulmonary vascular endothelium, which has potent vasoconstrictor effects and is known to facilitate the mitogenesis of vascular smooth muscle cells [8, 21]. Nitric oxide (NO) is an endothelium-derived vasorelaxant. Pulmonary vascular endothelial cells produce endogenous NO [12]. Increased pulmonary blood flow raises basal NO production [2]. Nitrate is one of the NO oxidation products and is regarded as an index of NO [19]. In several reports, plasma ET-1 and nitrate levels were found to be elevated in children with pulmonary hypertension (PH) associated with left-to-right shunt congenital heart defects [15, 16, 18, 22]. An imbalance among these factors, which is induced by endothelial cell injury, leads to pulmonary artery (PA) vasoconstriction and, ultimately, vascular remodeling [1, 10]. Plasma ET-1 levels were recently evaluated in only one study [7], and nitrate levels have not been previously studied in DS patients. We aimed to evaluate the plasma ET-1 and nitrate levels in DS patients with complete AVSD-associated PH and compare them to ND patients with complete AVSD-associated PH and control subjects.

Materials and methods

Study groups

We prospectively studied 20 patients (11 males, nine females) who had complete AVSD associated with PH who were evaluated during routine cardiac catheterization between February 2005 and March 2007 at our institution. To balance the ages in the two groups, only patients aged between 4 and 12 months were included in the study. The patients were divided into two groups according to the presence or absence of DS. Of the 20 patients, 12 were DS cases with proven diagnosis of trisomy 21 and eight were ND cases. Except for one patient in the DS group with Rastelli type B, all of the patient's echocardiographic diagnoses were complete AVSD with Rastelli type A. The patients associated with right outflow obstruction, such as pulmonary stenosis or tetralogy of Fallot, were excluded from the study. None of the patients underwent previous surgical procedures or had severe atrioventricular valve regurgitation, chronic pulmonary disease, or any other risk factor for the progression of pulmonary vascular disease. Ten age-matched healthy infants (five males, five females) with innocent heart murmur were included as the control group. Echocardiographic examination showed no evidence of cardiovascular anomalies or PH in the control group. None of the patients or control subjects had a known cause of increased ET-1 or NO, such as diabetes mellitus, systemic hypertension, sepsis, renal failure, or cardiogenic shock. All patients presented with heart failure and were under anticongestive therapy (digoxin, furosemide, and captopril). All drugs that were given for heart failure were discontinued for at least 24 h before catheterization. Informed consent was obtained from the parent(s) of each child enrolled in the study and the ethical committee of our hospital approved the study protocol.

Cardiac catheterization

Routine cardiac catheterizations and hemodynamic studies were performed with routine intravenous midazolam sedation. Pressure measurements were recorded using fluid-filled catheters connected to pressure transducers. Oxygen consumption was estimated based on age, sex, and heart rate, according to the method of LaFarge and Miettinen [9]. The pulmonary (Qp) and systemic (Qs) blood flow were calculated with the Fick equation and indexed for body surface area. The ratio of Qp/Qs blood flow, pulmonary (Rp) and systemic (Rs) vascular resistance, and the Rp/Rs ratio were calculated according to the standard formulas [17].

Blood sampling

Routine diagnostic catheterization was performed under spontaneous room-air respiration. Before angiography was performed, blood samples for ET-1 were obtained from the inferior vena cava, PA, pulmonary vein, and aorta during cardiac catheterization, all within 5 min, respectively. At the same time, one blood sample was obtained from a pulmonary vein for nitrate level measurement. Paired samples of arterial blood from the radial artery and venous blood from the antecubital vein of the contralateral extremity were obtained from control subjects at rest, and in the supine position. Blood gas was obtained from a pulmonary vein during catheterization.

Endothelin-1 assay

Blood samples were placed into ice-chilled tubes that contained EDTA-3K (3 mg/ml), aprotinin (Trasylol, Bayer) and 300 U/ml of blood. After centrifugation (3,000 rpm for 10 min at –4°C), the plasma was separated and stored at 40°C until ET-1 measurement. The concentration of ET-1 was measured by the ET-1 enzyme-linked immunosorbent assay (ELISA) method with commercially available kits (BI- 20052, Biomedica, Vienna, Austria).

Nitrate assay

Blood samples were centrifuged at 4,000 rpm for 10 min. Serum samples were then separated and stored at –70°C until they were assayed. The concentration of nitrate was determined using a procedure based on the Griess reaction

Table 1 Group comparisons of Down's (DS) and non-Down's (ND) syndrome patients with complete atrioventricular septal defects

Variables	DS patients (n=12)	ND patients (n=8)	Controls (n=10)	P-value
Age (months)	5 (4–8)	7 (4–12)	6 (4–12)	0.108
Gender (M/F)	7/5	4/4	5/5	0.907
Hemoglobin (g/dl)	11 (9–12)	12.3 (9.8–13.7)	12.4 (10.5–13.4	0.157
Oxygen saturation (%)	86 (72–95)	91 (67–95)	–	0.181
PH	7.32 (7.34–742)	7.38 (7.33–7.43)	–	0.851
PCO_2	36 (10–47)	35.2 (26–39)	–	0.910
HCO_3	19.5 (13–28)	20 (17–22)	–	0.970
Mean aortic pressure (mm Hg)	59 (42–70)	53 (44–72)	–	0.571
MPA pressure (mm Hg)	43.5 (36–50)	33.5 (29–44)	–	0.02
Qp/Qs	3.46 (1.35–4)	3.52 (2.27–4.8)	–	0.305
Qp	2.27 (1.41–2.54)	3.64 (2.29–5.45)	–	0.001
Rp (Wood u.m^2)	5.2 (2.56–6.77)	3.36 (1.95–3.9)	–	0.047
Rp/Rs	0.25 (0.11–0.40)	0.16 (0.10–0.27)	–	0.082
Associated heart defect	PDA: 2	PDA: 1		

Data are expressed as median and range (in parentheses)
MPA=mean pulmonary artery pressure; Qp/Qs=pulmonary to systemic flow ratio; Qp=pulmonary artery flow; Rp=pulmonary arterial resistance; Rp/Rs=pulmonary to systemic resistance ratio

method with commercially available kits (Nitric oxide colorimetric assay, 1–756–281, Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis

All statistical calculations were performed by using SPSS software (the Statistical Package for the Social Sciences, version 13; SPSS Inc., Chicago, IL). The values are given as median and range. Comparisons between groups were made by using Kruskal–Wallis and Mann–Whitney U-tests. Spearman's correlation coefficient was used to determine the relationships between variables. P-values<0.05 were considered to be statistically significant.

Results

Table 1 shows the characteristics of the patient groups and control subjects, and also the hemodynamic data in the patient groups. There were no significant differences between the two groups and control subjects in age, gender, and hemoglobin distribution. Pulmonary vein blood gases obtained during cardiac catheterization were not different between the two groups (P>0.05). The mean PA pressure and Rp were higher in the DS group than ND patients (P< 0.05). The Qp in ND patients was significantly higher than DS patients (P<0.001). The systemic artery oxygen saturation, mean aortic pressure, Qp/Qs, and Rp/Rs did not show any difference between DS and ND patients (P> 0.05). Patent ductus arteriosus (PDA) was associated in two DS and one ND patients. In the DS group, nine patients received digoxine + furosemide + captopril and three patients received digoxine + furosemide, whereas in the ND group, six patients received digoxine + furosemide + captopril and two patients recieved digoxine + furosemide (Table 1).

Table 2 shows the plasma ET-1 and nitrate levels at sequential sampling sites in the patient groups and in the peripheral venous and arterial samples of the controls. The systemic venous and pulmonary vein ET-1 concentrations of the study groups were compared to the peripheral venous

Table 2 Plasma endothelin-1 (ET-1) and nitrate levels in the two patient groups and the control subjects

Sample sites	DS (n=12)	ND (n=8)	Control subjects (n=10)	P-value
Pulmonary artery ET-1 (fmol/l)	1.62 (1.2–9)	1.9 (1.15–5.1)	–	0.851
Systemic artery ET-1 (fmol/l)	1.57 (1–9.6)	1.45 (1.25–4.7)	–	0.851
Pulmonary vein ET-1 (fmol/l)	1.72 (1.3–9.6)	2 (1.35–5.5)	0.19 (0.05–0.5)[a]	<0.001[†]
Systemic vein ET-1 (fmol/l)	1.55 (1–8.5)	1.77 (1.05–4.6)	0.17 (0.05–0.5)[b]	<0.001[†]
Pulmonary vein nitrate (μmol/l)	96 (48–320)	121 (30–244)	38 (32–42)[a]	<0.001[†]

Data are expressed as median and range (in parentheses)
[†] Statistical significance stems from the difference between the patient groups and controls, not between the DS and ND groups
[a] Peripheral arterial sample
[b] Peripheral venous sample

and arterial ET-1 levels of the control subjects. The pulmonary vein nitrate levels in the patient groups was compared to the arterial nitrate levels of the control subjects. PA (P=0.910), systemic artery (P=0.678), systemic vein (P=0.910), and pulmonary vein (P=0.970) ET-1 and pulmonary vein (P=0.521) nitrate levels of DS patients were not different from ND patients. Pulmonary vein and systemic venous ET-1, and pulmonary vein nitrate levels in the DS and ND patients were significantly higher than the control subjects' systemic arterial and venous values. In the DS group, the PA ET-1 and pulmonary vein nitrate levels were positively correlated with Qp (r: 0.832, P=0.001; r: 0.801, P=0.002), whereas it was inversely correlated with Rp (r: −0.669, P=0.017; r: −0.618, P=0.032). A correlation between the plasma ET-1 and nitrate levels was not obtained in the DS group. In the ND group, we found a positive correlation between the pulmonary vein nitrate levels with the ET-1 levels of PA (r: 0.778, P=0.023), pulmonary vein (r: 0.778, P=0.023), and systemic vein (r: 0.731, P=0.04). However, in this group, a correlation between the plasma ET-1 and vein nitrate levels with Qp and Rp and the mean PA pressure was not found.

Discussion

The reason why patients with DS and complete AVSD have an increased risk for the development of pulmonary vascular obstructive disease remains unclear. It is not known whether an increase in plasma ET-1 and nitrate levels are exceptional in the pathogenesis of DS patients with complete AVSD-associated PH. In this report, according to the underlying congenital heart disease, in a fairly homogenous group of patients with DS and ND, we have studied hemodynamic parameters and the plasma ET-1 and nitrate levels during routine cardiac catheterization. There is only one study in the literature that compares radial artery plasma ET-1 levels preoperatively in DS and ND patients with the left-to-right shunt [7]. Besides that, the present study is the first to evaluate the plasma nitrate levels in DS patients with complete AVSD-associated PH and compare them to ND patients.

The patients with DS often experience chronic upper airway obstruction due to macroglossia, recurrent pulmonary infection, thinned media of the pulmonary arterioles, and diminished number of alveoli, which are considered as high-risk factors for pulmonary vascular disease [5, 6, 20]. To assess the possible role of chronic airway disease in DS patients, blood gas was obtained from the pulmonary vein during catheterization and evaluated for hypercarbia and respiratory acidosis. None of the patients in the DS group showed evidence of compensated respiratory acidosis suggestive of chronic ventilatory problems (Table 1).

On the basis of hemodynamic data, Clapp et al. [5] reported that DS patients with complete AVSD have a greater degree of elevated pulmonary vascular resistance during the first year of life and a more rapid progression to fixed pulmonary vascular obstruction than patients without DS. Frescura et al. [6] correlated pulmonary resistance determined by hemodynamic study to pulmonary vascular disease based on histology and they concluded that the most severe pulmonary vascular disease was observed in patients with DS. However, other investigators did not find histological evidence that patients with DS were at a disadvantage [11]. The results of our study showed that the mean PA pressure and Rp in DS patients were significantly higher and Qp was lower than ND patients, and were consistent with these studies [5, 6].

Recently, Cappelli-Bigazzi et al. [3] evaluated endothelial cell function in patients with DS-associated left-to-right shunt by measuring the brachial flow velocity with an intravascular Doppler flow wire. They observed that DS patients had endothelial dysfunction, whereas the vascular smooth muscle function was intact. These authors suggested that their findings could be related to some specific alterations due to DS. Kageyama et al. [7] compared radial artery ET-1 in DS and ND patients preoperatively and found higher plasma ET-1 levels in DS patients compared to ND patients. In their report, the study groups had quite non-homogenous congenital heart defects and the Rp were not different between the two groups. The DS patients with complete AVSD-associated PH were exposed to persistent left-to-right blood flow shunts. This may cause shear stress on the endothelial cells in the pulmonary vasculature and impair the production of ET-1 and NO products, such as nitrates. All patients in this study had high pulmonary blood flow and PA pressure. The plasma ET-1 and nitrate levels were significantly higher in the DS and ND patients when compared to the controls. In the present study, the plasma ET-1 and nitrate levels in the DS patients with complete AVSD-associated PH were not different when compared to the group of ND patients (Table 2). In the DS group, the PA ET-1 and pulmonary vein nitrate levels were positively correlated with Qp and inversely correlated with high Rp. Although a correlation between the plasma ET-1 and nitrate levels was found in ND patients, no correlation was found in DS patients. This might be related to the higher Rp in DS patients.

The ET-1 and nitrate levels revealed no difference between the DS and ND patients with complete AVSD-associated PH. As our cohort was small, further studies are needed to evaluate the ET-1 and nitrate levels in DS patients to define their possible role in the pathogenesis of PH.

PH has a multifactorial pathophysiology. We measured only two mediators of PH in children with DS-associated complete AVSD. Besides specific mediators of pulmonary

endothelial cell function, such as thromboxane and prostacyclin metabolites, smooth muscle and fibroblast functions, which play a role in the pathogenesis of PH, may be investigated in further studies to discover why children with DS and left-to-right shunt show a more rapid progression towards PH compared to ND patients.

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