Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 59 (2010) 107 – 113
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Altered metabolisms of mediators controlling vascular function and enhanced oxidative stress in asymptomatic children with congenital portosystemic venous shunt

Hironori Nagasaka[a,*], Yoshiyuki Okano[b], Madoka Aizawa[c], Takashi Miida[d], Tohru Yorifuji[e], Go Tajima[f], Nobuo Sakura[f], Tomozumi Takatani[a], Yoshitami Sanayama[a], Kenji Sugamoto[g], Mitsufumi Mayumi[h], Kunihiko Kobayashi[i], Kenichi Hirano[j], Masaki Takayanagi[a], Hirokazu Tsukahara[h]

[a]*Division of Metabolism, Chiba Children's Hospital, Chiba 2666-0007, Japan*
[b]*Department of Pediatrics, Osaka City University Hospital, Osaka 545-8585, Japan*
[c]*Neonatal Intensive Care Unit, Chiba Children's Hospital, Chiba 2666-0007, Japan*
[d]*Department of Clinical Laboratory Medicine, Juntendo University, Tokyo 113-8421, Japan*
[e]*Department of Pediatrics, Kyoto University Hospital, Kyoto 606-8507, Japan*
[f]*Department of Pediatrics, Hiroshima University Hospital, Hiroshima 734-8551, Japan*
[g]*Division of Cardiology, Chiba Children's Hospital, Chiba 2666-0007, Japan*
[h]*Department of Pediatrics, Fukui University Hospital, Fukui 910-1193, Japan*
[i]*Department of Pediatrics, Hokkaido University, Sapporo 060-8638, Japan*
[j]*Department of Cardiovascular Medicine, Graduate School of Medicine, Osaka 565-0871, Japan*

Received 25 February 2009; accepted 14 July 2009

Abstract

Children with congenital portosystemic venous shunt (PSVS) are at risk for developing pulmonary hypertension, irrespective of the severity of portal hypertension or liver damage. Altered metabolisms of nitric oxide (NO) and endothelin-1 (ET-1), which are linked with oxidative stress and control vascular tone, might contribute to the vascular disturbance. This study examined 14 children (aged 1-5 years) with congenital PSVS lacking major liver damage and portal hypertension. Serum levels of nitrite/nitrate (NOx) as stable metabolites of NO, and of asymmetric dimethylarginine (ADMA) as an endogenous NO synthase inhibitor were determined, along with the plasma level of ET-1. Oxidative stress, which might affect the production of such mediators, was also examined using specific urinary and blood markers. The NOx levels were significantly lower in affected children than in the age-matched control group, although ET-1 levels were significantly higher than the control levels. In the affected children, the ADMA levels and ADMA/NOx ratios were higher, respectively, by 30% and 130% and showed significant positive correlations with the shunt ratios. Oxidative stress markers, including plasma thiobarbiturate reactive substances and urinary acrolein-lysine and 8-hydroxy-2′-deoxyguanosine, were significantly higher in affected children than in the control group, consistent with them being subjected to enhanced oxidative stress. These results suggest the presence of altered metabolisms of vascular mediators and enhanced oxidative stress in asymptomatic preschool children with congenital PSVS.
© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Congenital portal tract anomalies include portosystemic venous shunt (PSVS), which is characterized by flow through an anomalous direct bypass between intrahepatic or extrahepatic portal veins and systemic veins, but not through the liver [1-3]. In contrast to PSVS secondary to advanced liver diseases, congenital PSVS usually lacks portal hypertension and histologic findings of major liver damage; but it shows various clinical presentations including hyperammonemia, hypergalactosemia, prolonged coagulation, and cholestasis [1-5].

* Corresponding author. Tel.: +81 43 292 2111; fax: +81 43 292 3815.
E-mail address: nagasa-hirono@k2.dion.ne.jp (H. Nagasaka).

0026-0495/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.07.013

Recent developments in imaging techniques, together with the development of a neonatal mass-screening system, have facilitated the diagnosis of congenital PSVS; indeed, such cases have been increasingly detected. Concurrently, congenital PSVS patients have been shown to develop pulmonary hypertension, mostly after the age of 10 years [3,4,6,7]. However, the mechanisms underlying the vascular disturbances remain to be elucidated.

Nitric oxide (NO), a major vasodilator, is produced, along with L-citrulline, from L-arginine via the action of NO synthase (NOS; EC 1. 14. 13. 39) [8-10]. The L-citrulline is recycled to L-arginine through successive actions of argininosuccinate synthetase (EC 6. 3. 4. 5) and argininosuccinate lyase (EC 4. 3. 2. 1), forming the citrulline–NO cycle. Mammals have NOS of 3 distinct types: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). Of them, eNOS particularly plays important roles in the maintenance of vascular function.

Asymmetric dimethylarginine (ADMA), functioning as a competitor of L-arginine for NOS, is a major endogenous inhibitor of NOS [11-14]. Recently, ADMA has been revealed as an independent predictor for cardiovascular mortality [8]. Actually, ADMA is derived from the proteolysis of methylated arginine residues on various proteins, of which methylations are mediated by protein arginine *N*-methyltransferase (EC 2. 1. 1. 23), whereas the degradation of ADMA is mediated by dimethylarginine dimethylaminohydrolase (EC 3. 5. 3. 18) [12]. It has been suggested that oxidative stress increases protein arginine *N*-methyltransferase activity and decreases dimethylarginine dimethylaminohydrolase activity [13,14].

In contrast to NO, endothelin-1 (ET-1) is a major vasoconstrictor. It is often involved in the development or progression of vascular diseases [15,16].

Oxidative stress is also involved in vascular damage or vascular diseases [8,17-20]. The production of reactive oxygen species has been shown to be promoted by several factors supporting the elevation of blood pressure. Furthermore, enhanced oxidative stress suppresses NOS gene expression and enhances ET-1 synthesis [20].

Because the development of pulmonary hypertension is often fatal, we sought to examine vascular mediators in asymptomatic preschool children with congenital PSVS. We hypothesized that the risk of pulmonary hypertension might be associated with altered metabolisms of NO and ET-1 and enhanced oxidative stress in the affected children.

In this study, we measured the levels of NO, ADMA, and ET-1 in 14 preschool children with moderate PSVS flows through anomalous portal veins. We also evaluated oxidative stress using surrogate markers such as plasma thiobarbiturate reactive substances (TBARS) and urinary acrolein-lysine reflecting lipid peroxidation, and urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) reflecting oxidative DNA damage [21,22].

We described the possibility that long-standing abnormalities in such mediators might predispose these children to vasculopathies including pulmonary hypertension.

2. Subjects and methods

2.1. Subjects

Fourteen 1- to 5-year–old preschool children (8 boys, 6 girls) were enrolled. All 14 exhibited PSVS flow from anomalous intrahepatic and extrahepatic portal veins to systemic veins (Table 1). These cases included patent ductus venosus between the umbilical portion of the left portal vein and the inferior vena cava in 6 patients, an anomalous portal vein between the right portal vein and the right hepatic vein in 3 patients, an anomalous portal vein between the right portal vein and the middle hepatic vein in 1 patient, and an anomalous portal vein between the splenic vein and the left renal vein in 4 patients.

The diagnosis of congenital PSVS was made by abdominal ultrasonography and 3-dimensional computed tomography.

Table 1
Backgrounds and present data of patients with congenital PSVS

Pt no	Age	Sex	Portal anomalies	Shunt ratio (%)	NH_3 (μg/dL)	TBAs (μmol/L)	ALT (U/L)	AST (U/L)	PT (s)
1	1 y 1 m	M	Patent ductus venosus	33	66	39	22	30	12.1
2	3 y 5 m	F	Patent ductus venosus	49	69	57	31	31	13.3
3	2 y 2 m	M	Patent ductus venosus	45	75	104	49	53	13.5
4	1 y 3 m	M	Right PV → right HV	29	43	45	21	29	11.9
5	3 y 3 m	F	Right PV → middle HV	32	39	37	24	34	13.6
6	4 y 8 m	M	Right PV → right HV	55	71	51	49	54	13.9
7	2 y 1 m	M	Patent ductus venosus	32	59	29	26	29	11.5
8	4 y 2 m	M	Right PV → right HV	37	46	101	19	23	12.2
9	2 y 2 m	M	Patent ductus venosus	29	54	62	33	33	11.2
10	5 y 1 m	F	Patent ductus venosus	46	67	49	37	41	14.1
11	3 y 9 m	F	Splenic vein → renal vein	32	55	53	22	22	12.1
12	5 y 6 m	M	Splenic vein → renal vein	39	69	39	44	19	11.0
13	2 y 1 m	M	Splenic vein → renal vein	30	56	27	24	33	11.7
14	2 y 8 m	F	Splenic vein → renal vein	35	61	31	26	29	11.9
Control ranges	1-5 y	18F/18M			22-66	<20	<35	<35	<12

M indicates male; F, female; PV, portal vein; HV, hepatic vein; NH_3, ammonia; ALT, alanine aminotransaminase; AST, aspartate aminotransferase; PT, prothrombin time.

Abdominal ultrasonography revealed PSVS flows through anomalous direct bypasses toward the systemic veins in all affected children, but not through the liver or hemangioma. Three-dimensional computed tomography clearly revealed anatomical abnormalities in the subjects.

Metabolic screening tests at the age of 5 days revealed hypergalactosemia in all patients. Patients visited hospitals to receive follow-up examinations regarding hypergalactosemia at the ages of 19 to 27 days. During the follow-up examination, blood levels of total bile acids (TBAs) and transaminases were elevated; and plasma ammonia levels were slightly elevated in 6 of the 14 children. Immediately after the hypergalactosemia diagnosis, the children were started on lactose-free milk. Thereafter, their galactose levels normalized within a few months; but transaminase and TBA levels remained mildly elevated, without elevations in plasma procollagen type III peptide or type IV collagen, which are indicators of liver fibrosis. After the age of 1 year, nutritional intervention was discontinued; but their galactose levels remained normal. They did not develop the clinical presentations of portal hypertension, such as varices or splenomegaly, and were not subjected to internal or surgical treatment. They showed normal growth and development, and did not show abnormal findings on cardiac ultrasonography or in plasma brain natriuretic peptide levels, suggesting normal cardiac function.

Table 1 summarizes the clinical data for each of the 14 patients, including anomalies in portal vein and liver function. Patients with shunt ratios greater than 60% and considerably higher plasma ammonia levels were not enrolled because they were instead subjected to surgical therapies.

2.2. Study design

To estimate ET-1 and NO metabolisms, plasma ET-1 levels and serum levels of nitrite/nitrate (NOx) as stable metabolites of NO and ADMA were determined [10,12]. Plasma amino acids and ammonia levels were also determined.

To estimate oxidative stress status, plasma TBARS, urinary acrolein-lysine, and 8-OHdG were examined [20,21]. Furthermore, the activities of superoxide dismutase (SOD) and catalase, which are involved in antioxidant enzymatic systems, were determined in erythrocytes.

Blood was drawn from a peripheral vein in the morning after overnight fasting. The samples were transferred to chilled glass tubes containing EDTA with aprotinin (for ET-1) and were centrifuged promptly at 4°C; the supernatant plasma fraction was thus obtained. Serum and plasma were obtained for the determination of NOx, ADMA, amino acids, and TBARS. Erythrocytes were washed in cold 0.9% NaCl solution. Lysates for the determination of SOD and catalase activities were prepared by adding 100 μL of washed erythrocytes to 1 mL of distilled water, which was then frozen at −80°C until further analysis. Urine samples (10-45 mL) for oxidative stress markers were collected 0.5 to 2 hours before blood sample collection. For comparison, identical analyses were carried out in 32 (16 male, 16 female) age-matched, healthy controls.

This study protocol was approved by the relevant institutional review boards. The parents of all patients provided written informed consent before the start of the study.

2.3. Determination of the shunt ratio

In all patients, shunt ratios were determined by per-rectal portal scintigraphy with Tc-99m pertechnetate, as described previously [2]. Briefly, 111 MBq of Tc-99m pertechnetate (1 mL) was administered through a tube inserted into the upper rectum after administration of a laxative, following overnight cessation of solid intake. Subsequently, time-activity curves for the areas of the liver and heart were obtained every 4 seconds for 5 minutes. To determine the shunt ratio, the ratio of counts for the liver to those for the heart, integrated for 24 seconds, was calculated immediately after the appearance of the liver time-activity curve.

2.4. Assays for ET-1, NOx, and ADMA

Plasma ET-1 levels were measured using an ET-1 radioimmunoassay kit (Wako Pure Chemical Industries, Osaka, Japan). Serum NOx levels were measured using the Griess method, with a nitrate/nitrite colorimetric assay kit (Cayman Chemical, Ann Arbor, MI). Serum ADMA levels were determined using an enzyme-linked immunosorbent assay (ELISA) kit (DLD Diagnostika, Hamburg, Germany) [23,24].

2.5. Determination of plasma TBARS and urinary acrolein-lysine and 8-OHdG

Plasma TBARS concentrations were determined using a fluorometric assay, as described previously [25]. The concentrations of urinary acrolein-lysine and 8-OHdG were determined using the ACR-Lysine Adduct competitive ELISA kit (NOF, Tokyo, Japan) and the 8-OHdG Check competitive ELISA kit (Institute for the Control of Aging, Shizuoka, Japan), respectively [21,22].

2.6. Determination of SOD and catalase activity in erythrocytes

Superoxide dismutase activity was determined via spectrophotometry at 505 nm with the RANSOD kit (Randox Laboratories, Antrim, United Kingdom), as described previously [26]. Catalase activity was determined using the method described by Aebi et al [27]. Briefly, the decrease in absorbance at 240 nm in a reaction medium containing 20 mmol/L H_2O_2, 10 mmol/L potassium phosphate buffer (pH 7.0), and 0.1 to 0.3 mg protein per milliliter was monitored.

2.7. Statistical analyses

Differences between values of patients and those of controls were estimated using an unpaired Student *t* test. The

Table 2
Amino acids and vasoactivators

	Arg (nmol/L)	Cit (nmol/L)	ADMA (μmol/L)	NOx (μmol/L)	ADMA/NOx	ET-1 (pg/mL)
Patients (n = 14)	99 (22)	29 (7)	0.822 (0.198)*	22 (5)†	0.052 (0.019)‡	3.0 (0.5)†
Controls (n = 32)	105 (24)	27 (5)	0.631 (0.165)	30 (9)	0.023 (0.013)	1.9 (0.4)

Presented data are mean (SD) values. Arg. plasma arginine; Cit, plasma citrulline; ADMA, serum asymmetric dimethylarginine; NOx, serum nitrite/nitrate; ET-1, plasma, endothelin-1.
*$P < .05$, †$P < .01$, and ‡$P < .001$ vs age-matched controls.

relationship between each pair of parameters was estimated using Pearson correlation test. The regression lines were drawn with StatView (version 5.0J, Hulinks, Tokyo, Japan). P values < .05 were deemed to be statistically significant.

3. Results

3.1. Shunt ratios and plasma levels of ammonia and amino acids

Shunt ratios in the affected children were 29% to 55% (Table 1). Plasma ammonia levels in the affected children were significantly higher than those in the control group (53 ± 7 vs 33 ± 5 μg/dL, $P < .001$). Of the 14 affected children, 5 exhibited mildly elevated ammonia levels. However, amino acid profiles showed that plasma arginine and citrulline concentrations in the affected children were similar to those in the controls (Table 2). Other plasma amino acid levels, together with serum lipid levels and whole blood galactose and glucose levels, were also similar to the respective control levels (data not shown).

3.2. Blood NOx, ADMA, and ET-1 levels

Serum NOx levels in the affected children were significantly lower than those in the age-matched controls ($P < .01$). In contrast, ADMA levels and ADMA/NOx ratios in the affected children were 1.3-fold ($P < .05$) and 2-fold ($P < .001$) higher, respectively, compared with those in the controls. Their plasma ET-1 levels were also significantly higher than those of age-matched controls ($P < .01$, Table 2).

3.3. Oxidative stress markers and antioxidative enzyme activities in erythrocytes

Mean plasma TBARS, urinary acrolein-lysine, and urinary 8-OHdG levels were approximately 1.3-fold higher in the affected children than in the age-matched controls ($P < .01$, Table 2). Similarly, erythrocyte SOD and catalase activities were significantly higher in the affected patients than in the age-matched controls ($P < .05$, Table 3).

3.4. Correlations among parameters

In the control subjects, serum NOx levels showed strong positive correlations with plasma ET-1 levels ($P < .001$) and serum ADMA levels ($P < .001$), but no such correlation was found in the affected children (Fig. 1). However, the shunt ratio in the affected children was significantly correlated with ADMA levels ($r^2 = 0.693$, $P < .01$) and ADMA/NOx ratios ($r^2 = 0.855$, $P < .001$), but not with other parameters (Fig. 2).

The affected children were divided into 2 subgroups according to the shunt ratios and the plasma ammonia levels: a group with the shunt ratio less than 39% (n = 10) lacking hyperammonemia, except for 1 child whose ammonia level was at around the upper limit of the reference range, and a group with the shunt ratio greater than 39% (n = 4) presenting hyperammonemia. In the latter group, the shunt ratio showed strong correlations with the ADMA level ($r^2 = 0.986$, $P < .001$) and the ADMA/NOx ratio ($r^2 = 0.944$, $P < .001$) (Fig. 2). In the former group, such correlations were not significant.

4. Discussion

Clinical information on congenital PSVS has increased, and the complex nature of its pathophysiology has been demonstrated [1-3,6,7]. The clinical management of affected subjects, in particular young subjects, has focused on treating galactosemia, hyperammonemia, and liver dysfunction, as manifested by cholestasis and prolonged coagulation times. However, recently, a considerable number of adolescent or adult patients presenting with pulmonary hypertension have been reported, suggesting that long-standing congenital PSVS predisposes a patient to pulmonary hypertension [3,6,7]. Detailed and serial metabolic analyses in asymptomatic

Table 3
Oxidative stress markers and antioxidant enzyme activities in erythrocytes

	TBARS (mmol/L)	8-OHdG (ng/mg Cr)	ACR-lysine (nmol/mg Cr)	SOD (U/mg protein)	Cat (pmol/mg protein)
Patients (n = 14)	4.99 (1.01)†	25.7 (6.9)†	331 (60)†	1.19 (0.18)*	3.39 (0.33)*
Controls (n = 32)	3.93 (0.91)	18.8 (4.5)	255 (71)	1.06 (0.18)	2.96 (0.21)

Presented data are mean (SD) values. TBARS, plama thiobarbituric acid reactive substances; 8-OH-dG, urinary 8-hydroxy-2′-deoxyguanosine; ACR-lysine, urinary acrolein-lysine; SOD, superoxide dismutase activity in erythrocytes; Cat, catalase activity in erythrocytes.
*$P < .05$ and †$P < .01$ vs age-matched controls.

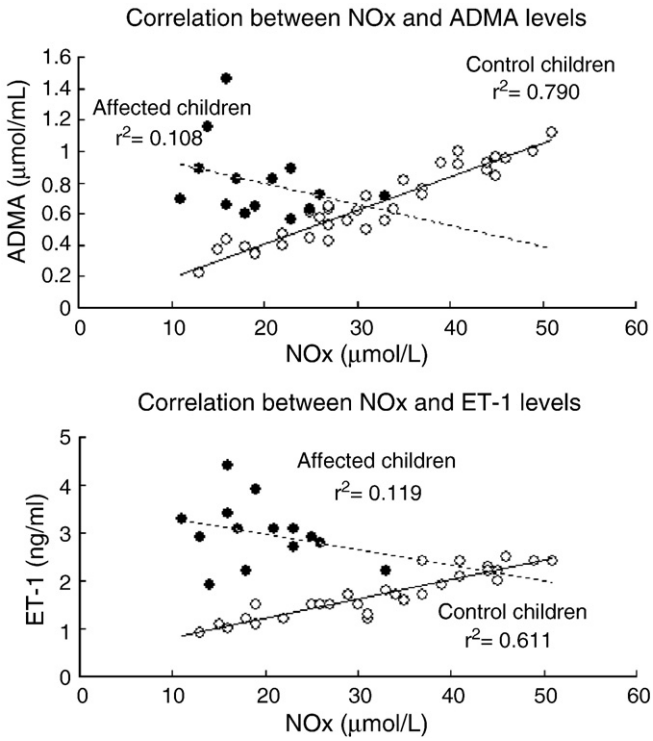

Fig. 1. Scatter graphs of plasma ET-1 and serum ADMA concentrations against serum NOx concentrations in affected and age-matched control children. Affected children, closed circles; age-matched controls, open circles. In the controls, serum NOx levels showed strong positive correlations with plasma ET-1 levels ($P < .001$) and serum ADMA levels ($P < .001$); but no such correlation was observed in the affected children.

patients and the correction of metabolic abnormalities are expected to contribute to prevention of this condition.

The results of this study suggest that, irrespective of liver damage, PSVS decreases NO production, increases ET-1 production, and enhances oxidative stress in affected subjects. Consequently, PSVS is likely to disturb the balance between vasodilating and vasoconstricting substances, favoring the latter even in preschool children. Changes in the cardiovascular system in patients with advanced liver disease complicating intrahepatic and extrahepatic PSVS have been investigated extensively. Abnormalities in vasoactivators, particularly those related to ET-1, are likely to contribute, at least in part, to the development of pulmonary hypertension in acquired PSVS [4,5]. During this study, our patients showed no apparent cardiopulmonary dysfunction. However, it is plausible that long-standing elevated ET-1 increases the likelihood of developing pulmonary hypertension in patients with congenital PSVS [15,16,28].

Serum ADMA levels and ADMA/NOx ratios both showed significant positive correlations with the PSVS shunt ratios, suggesting that PSVS promotes ADMA production. In particular, affected children with shunt ratios greater than 39% exhibited strong correlations. These results enabled us to speculate that patients having shunt ratios greater than 39% are at particularly high risk for developing vascular disturbances including pulmonary hypertension.

In this study, a 30% increase in serum ADMA levels was observed in the affected children; however, ADMA levels (0.822 μmol/L) remained well below the IC50 for each NOS isoform (eNOS, 3.9 μmol/L; nNOS, 1.8 μmol/L; iNOS, 2-10 μmol/L) [8,11,29,30]. Increased intracellular ADMA levels might be particularly relevant to lung diseases, including pulmonary hypertension, because the lungs exhibit high baseline concentrations of intracellular ADMA [31]. Small changes in serum ADMA might serve as indicators of greater changes in intracellular ADMA. Further studies are warranted to clarify this issue.

Similar to the other amino acids examined, plasma arginine levels in the affected patients were similar to those in the controls (99 ± 22 vs 105 ± 24 nmol/L, respectively). It might be difficult to estimate the influence of plasma arginine level on in vivo NO production because the K_m value of arginine for each NOS isoform (eNOS, <3.0 μmol/

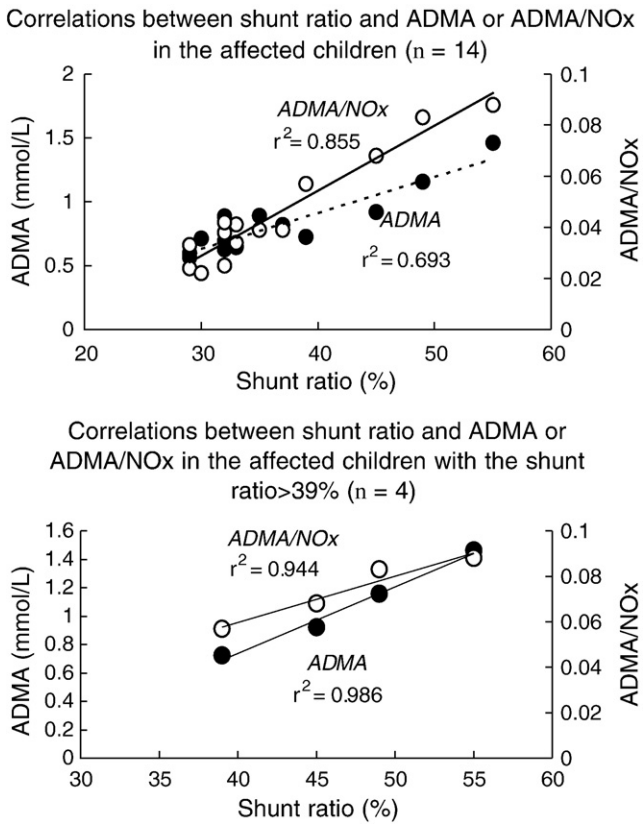

Fig. 2. Scatter graphs of serum ADMA concentrations and ADMA/NOx ratios against shunt ratios in affected children. ADMA, closed circles; ADMA/NOx ratio, open circles. In the 14 affected children, ADMA concentrations and ADMA/NOx ratios showed significant positive correlations with shunt ratios (ADMA, $P < .01$; ADMA/NOx ratio, $P < .01$). In the 4 children with shunt ratios greater than 39%, strong correlations were observed (ADMA, $r^2 = 0.986$, $P < .001$; ADMA/NOx ratio, $r^2 = 0.944$, $P < .001$). On the other hand, in the 10 children with shunt ratio less than 39%, such correlations were not significant.

L; nNOS, 1-3 μmol/L; iNOS, 3-30 μmol/L) is quite low compared with the plasma arginine and intracellular levels [8,32,33]. However, clinically, the administration of L-arginine and the subsequent rise in plasma arginine often improve vascular endothelial function.

The mechanisms underlying the observed increase in ET-1 levels also remain to be clarified. As a possible explanation, it has been proposed that the degeneration and elimination of ET-1 by the liver are reduced in PSVS [15]. Alternatively, PSVS or subsequently accumulated substances might stimulate the production of ET-1 in the liver, lungs, heart, and endothelium.

Oxidative stress markers were significantly higher in the affected children than in the age-matched healthy children. Although it is difficult to assess oxidative stress using these surrogate markers alone, concomitant increases in SOD and catalase activities in erythrocytes might indicate enhancement of the antioxidant enzymatic system in response to increased oxidative stress. In addition, blood levels of vitamin E and β-carotene, which function as antioxidants, decreased in the affected children (data not shown). Overall, the results of this study suggest that the affected children were subjected to enhanced oxidative stress.

Adverse effects of oxidative stress on vascular endothelial function have been demonstrated in many previous studies [17,19,20]. Furthermore, recent reports have demonstrated that enhancement of oxidative stress promotes ET-1 production in endothelial cells, but suppresses NO production by reducing NOS gene expression and/or by increasing ADMA production [13,14,18,20]. Consequently, enhanced oxidative stress might have induced the imbalance between NO and ET-1 in our patients.

In addition to the relaxation of vascular tone, NO exerts diverse vascular biological functions, such as inhibition of smooth muscle cell proliferation, platelet aggregation, and leukocyte adhesion, thereby preventing vascular diseases [8-10]. In contrast, ET-1 might also promote vascular inflammation and smooth muscle cell proliferation, thereby promoting vascular damage [15,16]. In this context, the long-standing imbalance between NO and ET-1 might be unfavorable for the vascular bed.

In summary, results of this study show that that congenital PSVS is accompanied by abnormalities in mediators controlling vascular function. More attention should be devoted to such mediators for clinical management of congenital PSVS.

References

[1] Uchino T, Matsuda I, Endo F. The long-term prognosis of congenital portosystemic venous shunt. J Pediatr 1999;135:254-6.

[2] Shiomi S, Sasaki N, Ikeda N, et al. Usefulness of per-rectal portal scintigraphy with Tc-99m pertechnetate for galactosemia in infants. Ann Nucl Med 1998;12:375-8.

[3] Ohno T, Muneuchi J, Ihara K, et al. Pulmonary hypertension in patients with congenital portosystemic venous shunt: a previously unrecognized association. Pediatrics 2008;121:e892-9.

[4] Hoeper MM, Krowka MJ, Strassburg CP. Portopulmonary hypertension and hepatopulmonary syndrome. Lancet 2004;363:1461-8.

[5] Krowka MJ, Swanson KL, Frantz RP, McGoon MD, Wiesner RH. Portopulmonary hypertension: results from a 10-year screening. Hepatology 2006;44:1502-10.

[6] Witters P, Maleux G, George C, et al. Congenital veno-venous malformations of the liver: widely variable clinical presentations. J Gastroenterol Hepatol 2008;23:e390-4.

[7] Marx M, Huber WD, Crone J, et al. Interventional stent implantation in a child with patent ductus venosus and pulmonary hypertension. Eur J Pediatr 2001;160:501-4.

[8] Forstermann U, Munzel T. Endothelial nitric oxide in vascular disease from marvel to menace. Circulation 2006;113:1708-14.

[9] Mori M, Gotoh T. Arginine metabolic enzymes, nitric oxide and infection. J Nutr 2004;134:2820S-5S.

[10] Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991;43:109-42.

[11] Kielstein JT, Tsikas D, Fliser D. Effects of asymmetric dimethylarginine (ADMA) infusion in humans. Eur J Clin Pharmacol 2006;62:39-44.

[12] Cook JP. ADMA: its role in vascular disease. Vasc Med 2005;10: S11-7.

[13] Sydow K, Munzel T. ADMA and oxidative stress. Atheroscler Suppl 2003;4:41-51.

[14] Lin KY, Ito A, Asagami T, et al. Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylaminohydrolase. Circulation 2002;106:987-92.

[15] Rubanyi GM, Polokoff MA. Endothelins: molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. Pharmacol Rev 1994;46:325-415.

[16] Little PJ, Ivey ME, Osman N. Endothelin-1 actions on vascular smooth cell functions as a target for the prevention of atherosclerosis. Curr Vasc Pharmacol 2008;6:195-203.

[17] Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. Nature 2006;444:875-80.

[18] Karaa A, Kamoun WS, Clemens MG. Oxidative stress disrupts nitric oxide synthase activation in liver endothelial cells. Free Radic Biol Med 2005;39:1320-31.

[19] Forstermann U. Oxidative stress in vascular disease: causes, defence mechanisms and potential therapies. Nat Clin Pract Cardiovasc Med 2008;5:338-49.

[20] Pechanova O, Simko F. The role of nitric oxide in the maintenance of vasoactive balance. Physiol Res 2007;56:S7-S16.

[21] Tamura S, Tsukahara H, Ueno M, et al. Evaluation of a urinary multiparameter biomarker set for oxidative stress in children, adolescents and young adults. Free Radic Res 2006;40:1198-205.

[22] Tsukahara H. Biomarkers for oxidative stress: clinical application in pediatric medicine. Curr Med Chem 2007;14:339-51.

[23] Tsukahara H, Ohta N, Tokuriki S, et al. Determination of asymmetric dimethylarginine, an endogenous nitric oxide synthase inhibitor, umbilical blood. Metabolism 2008;57:215-20.

[24] Shulze F, Wesemann R, Schwedhelm E. Determination of asymmetric dimethylarginine (ADMA) using a novel ELISA assay. Clin Chem Lab Med 2004;42:1377-83.

[25] Yagi K. Simple assay for the level of total lipid peroxides in serum or plasma. Methods Mol Biol 1998;108:101-6.

[26] McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 1969;244:6049-55.

[27] Aebi H, Wyss SR, Scherz B, Gross J. Properties of erythrocyte catalase from homozygotes and heterozygotes for Swiss-type acatalasemia. Biochem Genet 1976;14:791-807.

[28] Yoshibayashi M, Nishioka K, Nakao K, et al. Plasma endothelin levels in healthy children: high values in early infancy. J Cardiovasc Pharmacol 1991;17:S404-5.

[29] Nijveldt RJ, Teerlink T, Siroen MPC, Van Lambalgen AA, Rauwerda JA, Van Leeuwen PAM. The liver is an important organ in the metabolism of asymmetrical dimethylarginine (ADMA). Clin Nutr 2003;22:17-22.

[30] Siroen MPC, Van der Sijp JRM, Teerlink T, Van Schaik C, Nijveldt RJ, Van Leeuwen PAM. The human liver clears both asymmetric and symmetric dimethylarginine. Hepatology 2005;41:559-65.

[31] Zakrzewicz D, Eickelberg O. From arginine methylation to ADMA: a novel mechanism with therapeutic potential in chronic lung diseases. BMC Pulm Med 2009;9:5 [Review].

[32] Boger RH. Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, explains the "L-arginine paradox" and acts as a novel cardiovascular risk factor. J Nutr 2004;12:2842S-7S.

[33] Tsikas D, Boger RH, Sandmann J, Bode-Boger SM, Frolich JC. Endogenous nitric oxide synthase inhibitors are responsible for the L-arginine paradox. FEBS letters 2000;478:1-3.