Endothelin-1 plays an important role in the Fontan circulation

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Introduction and Objective

The Fontan procedure failed in some patients despite fulfilment of the usual hemodynamic criterion. Several studies revealed that an increased medial thickness of the small pulmonary arteries was observed in patients whose pulmonary arterial pressure (PAP) and PVR were normal. Significant medial hypertrophy with proliferation of vascular smooth muscle cells and the overexpression of endothelin-1 (ET-1) in the intra-acinar pulmonary arteries of the autopsy lung tissues of failed Fontan patients. ET-1 is one of the potent endogenous vasoconstrictors. Yamagishi et al. noted that the plasma ET-1 level in Fontan patients correlated with the central venous pressure. Thus, we hypothesized that the endothelin system may play an important role in maintaining vasoconstriction of the pulmonary artery, which may subsequently promote vascular remodeling in patients with SV physiology.
To evaluate whether endothlin-1 (ET-1) plays an important role in the Fontan circulation.

Conclusions

- Medial hypertrophy and the overexpression of ET-1 in the pulmonary arteries may induce failure of the Fontan circulation.
- There is an association between ET-1 and SV physiology in maintaining the Fontan circulation.

Patients and Methods

1) we measured the serum ET-1 levels taken from the pulmonary artery in 32 patients with single ventricle (SV) physiology and compared this group with 28 patients with biventricular physiology (BV).

2) Twelve patients (Glenn circulation; n = 7, Fontan circulation; n = 5) were evaluated using lung histopathological and immunohistochemical studies.

- The medial thickness (D) value at R = 100 μm ($D_{R = 100\mu m}$) was computed using the ImageJ software program (http://www.rsb.info.nih.gov/ij/).
- Sections were immunostained with anti-ET-1(Abnova, H00001906-M01, Taiwan) to evaluate proliferation of the media.
- For 10 of these patients, quantitative real-time PCR analyses of ET-1, endothelin receptor type A and type B were performed.

Quantitative RT-PCR was performed with the LightCycler96 system (Roche Diagnostics, Basel, Switzerland) using the following PCR conditions: 40 cycles 95℃ for 10 s, 52℃ for 10 s and 72℃ for 10 s. For ET_BR, the PCR conditions were 40 cycles 95℃ for 20 s, 55℃ for 20 s and 72℃ for 20 s.
The oligonucleotides used for PCR amplification were as follows: ET-1 forward, GAGGCTATGGCTTCAG ACAGG and reverse, AAGTTCAGAGGAACACCTAAGACAAAC; ET_AR forward, TGATAGCCAGTCTTGCCCTT and reverse, CTGTACCTGTCAACACTAAGAGCG; ET_BR forward, TGCTTGCTTCATCCCGTTCA and reverse, T TCCCGTCTCTGCTTTAGGTGA; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward, TGTGTCC GTCGTGGATCTGA and reverse, TTGCTGTTGAAGTCGCAGGAG. The relative mRNA levels were normalized to GAPDH.

Table 1. Clinical characteristics of the patients

Case	Sex	Diagnosis	Operations	Outcomes
1	F	SV, PA, TAPVC, pulmonary CoA, Asplenia	Central PA plasty + SP shunt (12 days) BDG (10 monthss) TCPC + lung biopsy (3 years)	Surviving
2	M	SV, PA, pulmonary CoA, apico-caval juxtaposition	Central PA plasty + SP shunt (10 days) bilateral BDG + ASD enlargement (3 months) TCPC + lung biopsy→take down (2 years)	Obstruction of extracardiac conduit→ Fontan take down Surviving
3	F	HLHS variant	Bilateral PAB (11 days) Norwood (3 months) Bilateral BDG (11 months) Arch repair + ASD enlargement + MVP (1 year) CRT + lung biopsy (1 year)	Died of ventricular dysfunction (3 years)
4	F	DORV, MS (hypo LV)	PAB (3 days) ASD enlargement (9 months) Bilateral BDG + TVP (2 years) TCPC + lung biopsy (3 years)	Surviving
5	M	SV, PS, TAPVC, CAVV, Asplenia,	TAPVC repair (19 days) BT shunt (1 year) BDG (1 year) CAVV replacement + lung biopsy (6 years)	Pulmonary hypertenstion due to numerous collateral arteries Surviving
6	F	TGA, PS, multiple VSD	BDG + DKS (6 months) TCPC + lung biopsy (1 year)	Surviving
7	M	Fallot, AVSD, multiple VSD, 21-trisomy	Central PA plasty + SP shunt (2 months) BDG (3 years) TCPC + lung biopsy → died (5 years)	Died of congestive heart failure (5 years)
8	F	TA	BT shunt (3 months) APC Fontan (4 years) TCPC conversion + lung biopsy (27 years)	Surviving
9	M	TA	SP shunt + ASD enlargement (1 month) BDG (1 year) TCPC (2 years) Plication of diaphragm + lung biopsy (4 years)	Surviving
10	F	SV, PS, TAPVC, apico-caval juxtaposition, Asplenia	TAPVC repair (0 day) BDG (8 months) TCPC (2 years) Release of lt.PVO + lung biopsy (3 years)	Surviving
11	F	SV, PS,CAVV, TAPVC, Asplenia	BT shunt (1 month) BDG + CAVV plasty (9 months) CAVV replacement (10 months) TCPC (2 years) Redo CAVV replacement + lung biopsy (10 years)	Surviving
12	M	SV, PS,CAVV, TAPVC, Asplenia	Bilateral BDG + CAVV plasty (7 months) CAVV replacement (8 months) TCPC (2 years) Redo CAVV replacement + PMI (3 years) 3rd CAVV replacement+lung biopsy (12 years)	Surviving

Table 2. Hemodynamic and pathological data of the patients

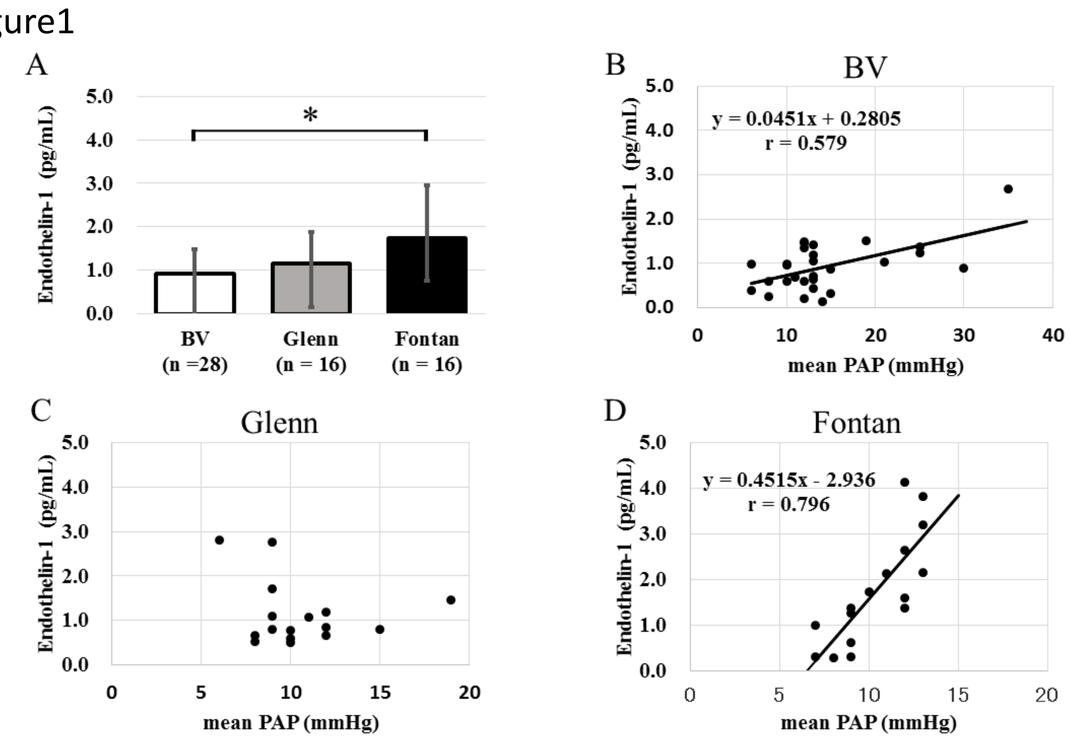
Case	CVP (mmHg)	Mean PAP (mmHg)	PCWP (mmHg)	PVR (units × m^2)	$D_{R = 100\mu m}$ (μm)	RT-PCR ET-1
1	13	12	8	1.3	4.9	-
2	7	6	5	1.7	23.9	1.26
3	13	13	10	2.4	12.7	0.98
4	17	17	-	2.7	5.6	1.03
5	24	24	20	2.5	19.0	1.72
6	16	15	-	2.7	13.0	0.82
7	13	12	13	1.5	19.9	1.87
8	-	-	-	-	8.4	-
9	8	7	6	1.3	5.8	0.70
10	-	-	-	-	22.1	0.52
11	16	16	12	1.5	14.2	1.55
12	22	22	4	0.5	5.4	1.45

Results

1)The serum ET-1 levels in the patients in the Fontan, Glenn and BV groups were 1.7 ± 1.2, 1.1 ± 0.6, and 0.9 ± 0.5 pg/mL, respectively. The serum ET-1 level positively correlated with the mean pulmonary artery pressure in Fontan circulation ($p < 0.01$) (Figure1).

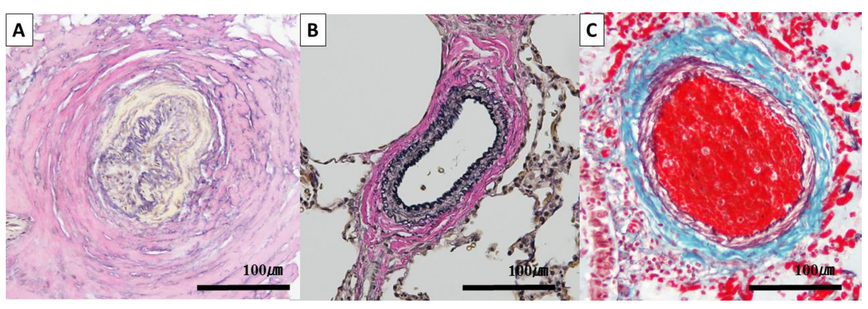
2)In patients with failed Fontan circulation, the pulmonary arteries exhibited severe medial hypertrophy and the overexpression of ET-1 in the endothelium and media(Table2, Figure2 and 3). Quantitative real-time PCR analyses also confirmed that the mRNA expression of ET-1 was increased in patients with failed Fontan circulation(Figure4).

Figure1



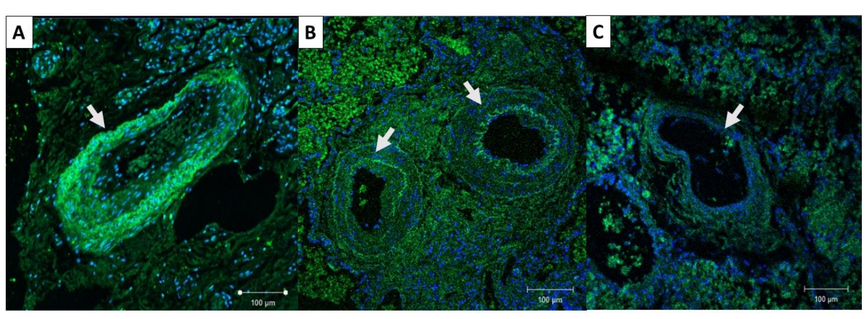
Analyses of serum endothelin-1 (ET-1) levels.
A: The mean ET-1 level in the Fontan group was significantly higher than that in the bi-ventricular (BV) group. Error bars represent 95% confidence interval. *$p < 0.01$.
B, C, D: The relationship between the mean pulmonary artery pressure and serum ET-1 level of the pulmonary artery in the BV group (B), Glenn group (C) and Fontan group (D), respectively. The serum ET-1 level was strongly correlated with the mean pulmonary artery pressure in patients with Fontan circulation.

Figure 2



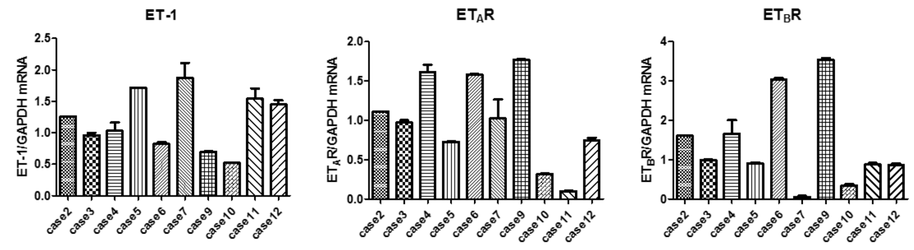
A: Elastica van Gieson staining of the pulmonary artery in a patient who did not undergo successful Fontan operation showed both severe intimal and medial hypertrophy (case 2).
B: Elastica van Gieson staining of pulmonary arteries in a patient with a satisfactory clinical course showed slightly hypertrophic media (case 6).
C: Masson's trichrome staining of the pulmonary arteries in normal control showed thin media.

Figure 3



A: In a patient who did not undergo successful Fontan operation, both the endothelium and media were strongly stained (case2).
B: In a patient with a satisfactory clinical course, ET-1 was observed in the endothelium (case6).
C: In the normal control, the media and endothelium was barely stained. Arrowheads demonstrate pulmonary arterial media.

Figure 4



A: ET-1, **B**: ET_AR, **C**: ET_BR. Five patients (cases 2, 5, 7, 11 and 12) showed high ET-1 expression levels. Two patients (cases 6 and 9) showed high ET_AR and ET_BR expression levels.

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