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Association between levels of anti-angiogenic isoform of vascular endothelial growth factor A and pulmonary hypertension☆

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ABSTRACT

Backgrounds: Pulmonary hypertension (PH) is characterized by elevated pulmonary arterial pressure due to vasoconstriction and remodeling of the pulmonary microvasculature. Vascular endothelial growth factor (VEGF) is a key contributor for angiogenesis and vasculogenesis. VEGF165b is recently identified as an anti-angiogenic splicing variant of VEGF. The aim of this study was to examine the association between circulating levels of VEGF165b in PH patients under consideration with classifications of PH.

Methods and results: We measured plasma levels of VEGF165b in the PH group (pulmonary artery hypertension [PAH], n = 26; chronic thromboembolic pulmonary hypertension [CTEPH], n = 13) and control group (n = 30). Circulating levels of VEGF165b were higher in PH group than controls (97.1 vs. 53.3 pg/ml, $P < 0.01$). The multiple regression analysis demonstrated that the independent factor to determine the plasma levels of VEGF165b was the presence of PH ($P = 0.04$). Next, we focused on differences in $VEGF_{165}b$ levels and classifications of PH. Plasma VEGF165b level was higher only in idiopathic PAH (n = 9) than in control (137.1 vs. 53.3 pg/ml, $P < 0.01$), but not in PH related to collagen disease (n = 7), congenital heart disease (n = 10) and CTEPH (n = 13).

Conclusions: We demonstrated associations between circulating levels of $VEGF_{165}b$ and classifications of PH. VEGF165b, anti-angiogenic isoform, might contribute to the pathophysiology in PH, especially in idiopathic PAH. The level of plasma VEGF165b might be a novel marker that reflects the pathological conditions in patients with PH.

1. Introduction

Pulmonary hypertension (PH) is characterized by elevated pulmonary arterial pressure due to vasoconstriction and remodeling of the pulmonary microvasculature, and leads to right ventricular failure and decreasing of exercise tolerance [1]. Development of PH involves the complex interactions of multiple effectors, and updated classification of PH divided five groups based on each disease mechanism [2]. In group 1, there is a primary form of PH, defined as idiopathic pulmonary artery hypertension (PAH), while the secondary disease state, including congenital heart disease with systemic-to-pulmonary shunts and collagen disease, forms pulmonary vascular remodeling resembling idiopathic PAH in histological changes. Chronic thromboembolic pulmonary hypertension (CTEPH), classified as group 4, is caused by organized multiple pulmonary thrombosis [3]. In this way, trigger and progression mechanisms of PH are different by each of origin, however, generation and progression mechanisms of idiopathic PAH have remain unclear.

Vascular endothelial growth factor (VEGF) is a key contributor for angiogenesis and vasculogenesis, and the VEGF family includes VEGF-A, VEGF-B, VEGF-C and VEGF-D, being the VEGF-A the most important member of the family. Some reports of clinical and basic research revealed that plasma VEGF level and lung expression levels of VEGF and VEGF receptor (VEGFR) were elevated in response to hypoxia [4,5]. In animal model, the blockade of VEGF-A receptor with Sugen5416 can induce PH [6], and $VEGF_{165}b$ is a specific isoform of VEGF-A. Alternative splicing and proteolytic processing of VEGF-A produce various isoforms [7]. The particular splicing event in the terminal exon creates two whole families of isoforms, $VEGF_{xxx}$ isoforms are pro-angiogenic family, on the other hand, $VEGF_{xxx}b$ isoforms are anti-angiogenic family. $VEGF_{165}b$, the major anti-angiogenic isoform, is the first member of the $VEGF_{xxx}b$ family [8], and inhibits migration and proliferation of endothelial cells and physiological angiogenesis [9]. Since $VEGF_{165}b$ might have similar effect to Sugen and contribute to pathological condition of PH, we hypothesized that circulating levels of $VEGF_{165}b$ would be increased in PH patients and varied depend on the etiology of PH. Therefore, the purpose of this study was to examine the circulating levels of $VEGF_{165}b$ in

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patients with PH and subjects without PH, and to compare differences in $VEGF_{165}b$ levels among each etiology of PH.

2. Methods

2.1. Subjects and study protocol

We enrolled 39 consecutive patients with PH (mean age, 52.6 years old, 26 in PAH [group 1], and 13 in CTEPH [group 4]), who admitted to Fukushima Medical University Hospital for diagnosis and treatment of PH between January 2010 and September 2014. These patients had over 25 mm Hg of mean PAP based on the right heart catheterization in stable condition. PAH patients were consisted of 9 idiopathic PAH, 7 collagen disease and 10 congenital heart disease with systemic to pulmonary circulation shunt flow (8 atrial septal defect, 2 ventricular septal defect). Thirty normal subjects, with no abnormalities detected on physical examination, electrocardiogram, chest x-ray, and echocardiography, served as controls (mean age, 59.0 years old). We compared clinical characteristics, echocardiographic parameters, and laboratory data including plasma levels of $VEGF_{165}b$ between the groups. Hypertension was defined as the recent use of antihypertensive drugs, or systolic blood pressure ≥ 140 mm Hg, and/or diastolic blood pressure ≥ 90 mm Hg. Diabetes was defined as the recent use of insulin or anti-diabetic drugs, fasting blood glucose >126 mg/dl, and/or hemoglobin A1c >6.5%. Dyslipidemia was defined as the recent use of cholesterol-lowering drugs, triglyceride >150 mg/dl, low-density lipoprotein cholesterol >140 mg/dl, and/or high-density lipoprotein cholesterol <40 mg/dl. Blood sample was obtained and echocardiography and cardiac catheterization were performed at stable condition during hospitalization. Written informed consent was obtained from all study subjects. Our study complies with the 1975 Declaration of Helsinki, and the study protocol was approved by the ethical committee of Fukushima Medical University.

2.2. Echocardiography

Echocardiography of patients with PH was performed by a blinded, experienced echocardiographer using the standard techniques [10]. The echocardiographic parameters investigated included left ventricular end-diastolic volume (LVEDV), left ventricular ejection fraction (LVEF), tricuspid valve regurgitation pressure gradient (TR-PG), inferior vena cava (IVC) diameter, right ventricular end-diastolic diameter (RVEDD), and right ventricular fractional area change (RV-FAC) [11]. LVEF was calculated using a modification of Simpson's method. RV-FAC, defined as (end diastolic area − end systolic area) / end diastolic area × 100, is a measure of RV systolic function [11]. All recordings were performed on ultrasound systems (ACUSON Sequoia, Siemens Medical Solutions USA, Mountain View, CA, USA).

2.3. Blood sample examinations

Plasma BNP concentrations were measured using a commercially available radioimmunoassay specific for human BNP (Shionoria BNP kit, Shionogi, Osaka, Japan). The measurement of high sensitive C-reactive protein (hs-CRP) was performed using the CRP-Latex (II) immunoturbidimetric assay (Denka Seiken, Tokyo, Japan). Estimated glomerular filtration rate (eGFR) was measured by the Modification of Diet in Renal Disease (MDRD) formula. Plasma levels of $VEGF_{165}b$ were measured using the commercially available ELISA kit (My Biosource, Inc. San Diego, CA).

2.4. Right heart catheterization

Right heart catheterization in PH patients was performed during hospitalization by an attending physician with standard techniques. We investigated the pressure of right atrium, right ventricle, pulmonary artery, pulmonary capillary wedge pressure, and cardiac index.

2.5. Statistical analysis

Categorical variables are expressed as numbers and percentages. The chi-square test was used for comparisons of categorical variables. Normally distributed data are presented as mean ± SD, and non-normally distributed data are presented as median (inter-quartile range). Data of the two groups were compared using the independent Student's *t*-test for normally distributed data and the Mann–Whitney U test for non-normally distributed data. Comparisons among three groups were performed using analysis of variance followed by Bonferroni's post-hoc test and the Kruskal-Wallis test, as appropriate. We performed multiple regression analysis allowing for interaction between plasma levels of $VEGF_{165}b$ and possible confounding factors. Parameters with statistical significance in the univariate analysis ($P < 0.05$) were included in the multivariate analysis. A value of $P < 0.05$ was considered statistically significant for all comparisons. Statistical analysis was performed with a standard statistical program package (SPSS ver. 21.0, IBM, Armonk, NY, USA).

3. Results

Comparisons in clinical characteristics between control subjects and PH patients are shown in Table 1. The prevalence of male gender was lower, body mass index was smaller in PH patients than in control. Plasma levels of B-type natriuretic peptide and $VEGF_{165}b$ were significantly higher in PH patients than in control subjects ($P < 0.01$). In echocardiographic data, although LVEDV was smaller ($P \le 0.01$) and LVEF was higher ($P \le 0.05$), TR-PG was greater ($P \le 0.01$), RVEDD was larger ($P \le 0.05$), and RV-FAC was lower ($P \le 0.01$) in PH patients than in control (Table 1). As shown in Table 2, the multiple regression analysis demonstrated that the independent factor to determine the plasma levels of $VEGF_{165}b$ was the presence of PH ($\beta = 29.83$, $P \le 0.01$).

Next, we focused on differences in $VEGF_{165}b$ levels and classifications of PH. All PH patients were divided into two groups based on the classification of PH: PAH (group 1) and CTEPH (group 4). Echocardiographic data did not show significant differences between two groups. $VEGF_{165}b$ was significantly higher in PAH group than in control, but not in CTEPH (Fig. 1A).

Additionally, we divided all PAH patients into three groups based on the primary disease: idiopathic PAH, PAH related to collagen disease, and congenital heart disease. Comparisons of clinical characteristics of all PH patients are shown in Table 3. The patients with idiopathic PAH and PAH related to collagen disease were younger than those with CTEPH. World health organization (WHO) functional class of PH severity, laboratory data and echocardiographic parameters did not show significant differences among groups (Table 3). Right heart catheterization demonstrated that mean right atrial pressure, mean pulmonary artery pressure, and cardiac output did not show significant differences among the etiologies of PH (Table 3). The use of warfarin was extremely higher in the CTEPH patients (100%) and lower in the PAH patients with collagen disease (14.3%) among all groups ($p \le 0.01$). As for vasodilators for PH, there was no difference in the use of prostanoids among the groups, whereas the use of phosphodiesterase-5 inhibitors or endothelin receptor antagonists was higher in the idiopathic PAH patients (77.8% and 88.9%, respectively) and lower in the CTEPH patients (15.4% and 23.1%, respectively).

Interestingly, plasma $VEGF_{165}b$ level was significantly higher only in idiopathic PAH than in control (137.1 vs. 53.3 pg/ml, $P < 0.001$), but not in PH related to collagen disease and congenital heart disease (Fig. 1B).

Table 1
Comparisons of clinical characteristics between control and PH patients.

	Control ($n = 30$)	PH ($n = 39$)	*p* value
Age (years)	59.0 ± 14.3	52.6 ± 15.6	N.S.
Gender (male/female)	19/11	9/30	<0.01
Body mass index (kg/m^2)	27.3 ± 7.9	22.6 ± 3.6	<0.01
Hypertension (n, %)	19 (63%)	17 (44%)	N.S.
Diabetes mellitus (n, %)	10 (33%)	12 (31%)	N.S.
Dyslipidemia (n, %)	15 (50%)	21 (54%)	N.S.
Blood sample data			
Hemoglobin (g/dl)	14.0 ± 1.5	13.4 ± 2.6	N.S.
Estimated GFR (ml/min/1.73 m^2)	71.9 ± 23.7	72.1 ± 21.1	N.S.
BNP[a] (pg/ml)	17.3 (24.1)	124.7 (300.1)	<0.01
hs-CRP[a] (mg/dl)	0.05 (0.53)	0.13 (0.95)	N.S.
$VEGF_{165}b$ (pg/ml)	53.3 ± 13.4	97.1 ± 56.2	<0.01
Echocardiographic data			
LVEDV (ml)	91.0 ± 37.5	56.1 ± 28.3	<0.01
LVEF (%)	62.7 ± 7.0	68.3 ± 8.2	<0.05
TR-PG (mm Hg)	11.2 ± 10.6	71.0 ± 25.9	<0.01
IVC (mm)	12.7 ± 3.0	15.3 ± 5.3	<0.05
RVEDD (mm)	29.8 ± 6.2	37.3 ± 5.8	<0.05
RV-FAC (%)	47.4 ± 9.2	32.7 ± 13.0	<0.01

PH, pulmonary hypertension; GFR, glomerular filtration rate; BNP, brain natriuretic peptide; hs-CRP, high-sensitive C-reactive protein; VEGF, vascular endothelial growth factor; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; TR-PG, tricuspid regurgitation pressure gradient; IVC, inferior vena cava; RVEDD; right ventricular end-diastolic diameter, RV-FAC; right ventricular fractional area change.
[a] Skewed data are reported as median (inter-quartile range).